CELL MECHANICS

1. Introduction

2. Physical principles

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

In the previous lesson we discussed about: thermical forces and diffusion

- **Boltzmann's law**

  describes how the probability of a molecule being in a certain energy state depends on the surrounding temperature

- **Principle of Equipartition of Energy**

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

- **Einstein relation**

  relates the diffusion coefficient of a molecule to its drag coefficient

- **Autocorrelation function, Power Spectrum**

  allow to analyse the statistical properties of the motion affected by thermal forces and deduce molecular properties of a mechanical system, e.g. stiffness, mass, damping from thermal motion

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
- 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

By chemical forces we mean forces arising from formation of intermolecular bonds.

Ex: protein-ligand, protein – protein contact. As energetically favorable contacts are made, the protein may become stretched or distorted from its equilibrium conformation.

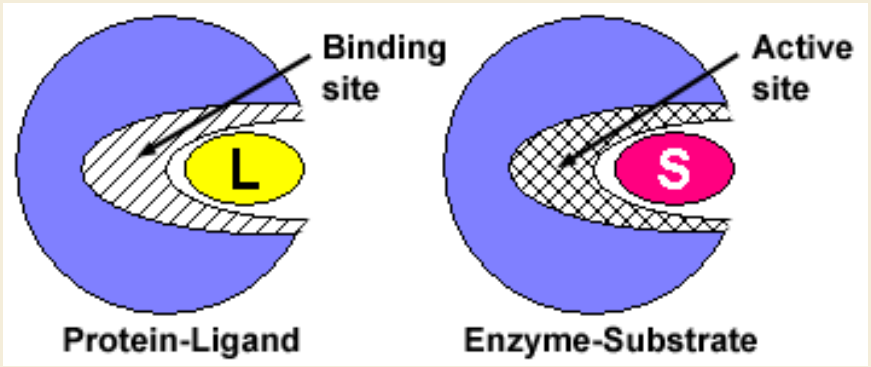
Force	Model	Basis of Attraction	Energy (kJ/mol)	Example
Ion-dipole		Ion charge–dipole charge	40–600	$Na^+ \cdots O$ (H, H)
H bond	$\delta- \quad \delta+ \quad \delta-$ $-A-H\cdots:B-$	Polar bond to H–dipole charge (high EN of N, O, F)	10–40	$:\ddot{O}-H\cdots:\ddot{O}-H$ (H, H)
Dipole-dipole		Dipole charges	5–25	I—Cl···I—Cl
Ion–induced dipole		Ion charge–polarizable e^- cloud	3–15	$Fe^{2+}\cdots O_2$
Dipole–induced dipole		Dipole charge–polarizable e^- cloud	2–10	H—Cl···Cl—Cl
Dispersion (London)		Polarizable e^- clouds	0.05–40	F—F···F—F

Comparison of intermolecular forces

electrostatic interactions

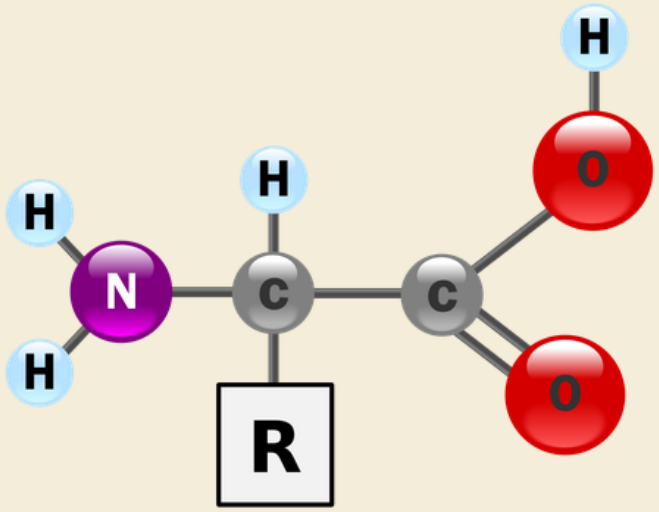
For conversion:1 kJ/mol ~ 0.4 KT/molecule

protein-ligand schematic



Chemical bonds in proteins

Amino Acids – the structural units that make up proteins



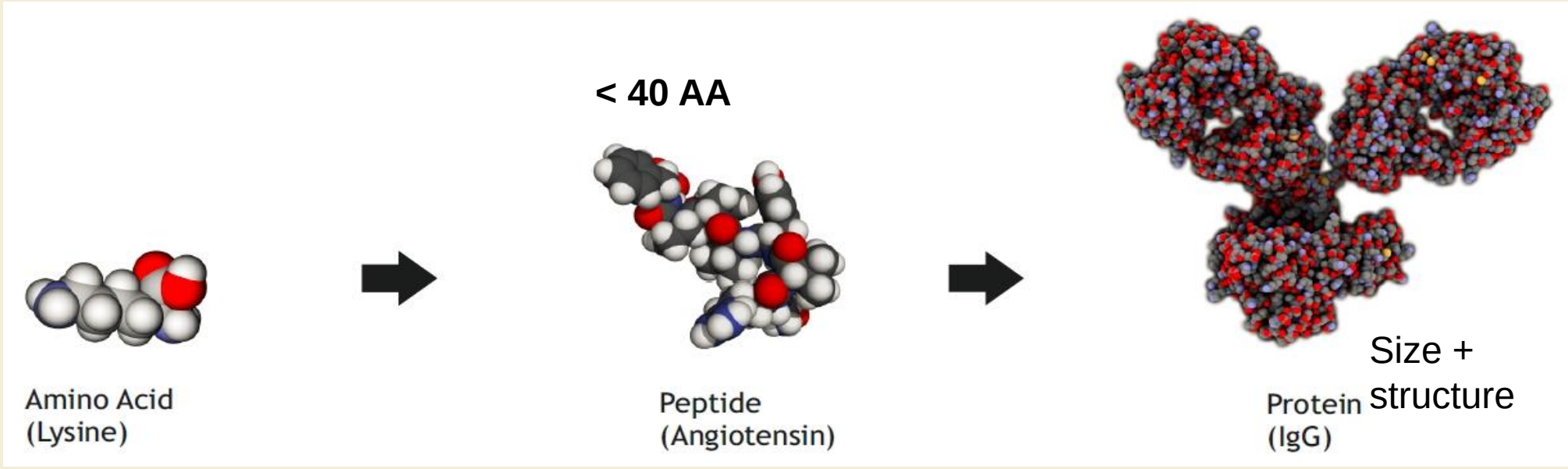
chemical structure

Size: 0.4 – 1 nm
Molecular Mass: 75 – 205 Da
Smallest AA: glycine; Biggest AA: thryptophan

In nature > 100. The human body is able to synthesize 11 of the **20 amino acids.**.

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 conponent that differs between amino acids.

Amino Acid AA **Peptide** **Protein**



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 ?

CLASSES / LEVELS OF PROTEIN STRUCTURE

Primary Structure = sequence of amino acids

3-letter code

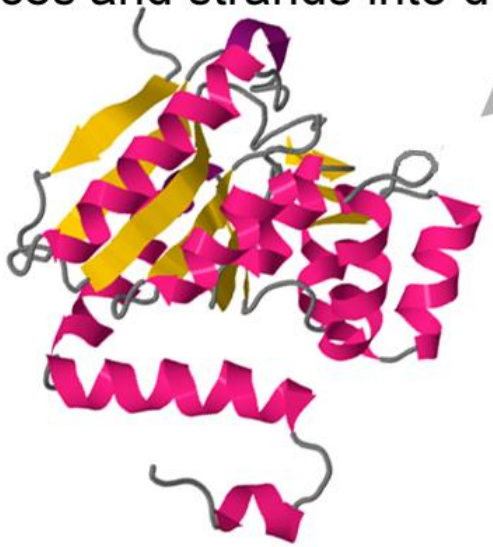
Lys-Thr-Tyr-Phe-Pro-His-Phe-Asp-Leu-Ser-His-**Gly** ...

1-letter code

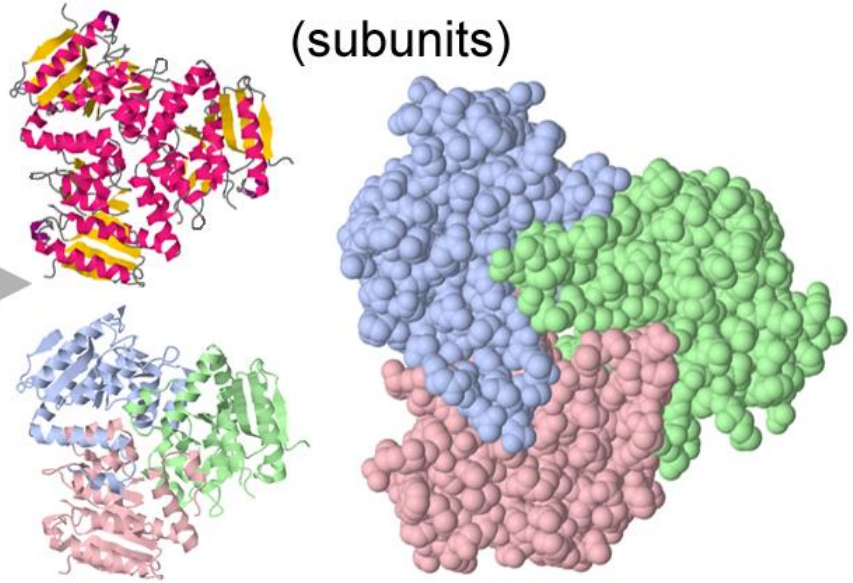
KTYFPHFDLSH**G**

Secondary Structure = **alpha helices**, **beta strands**

Tertiary Structure = fold helices and strands into domains



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

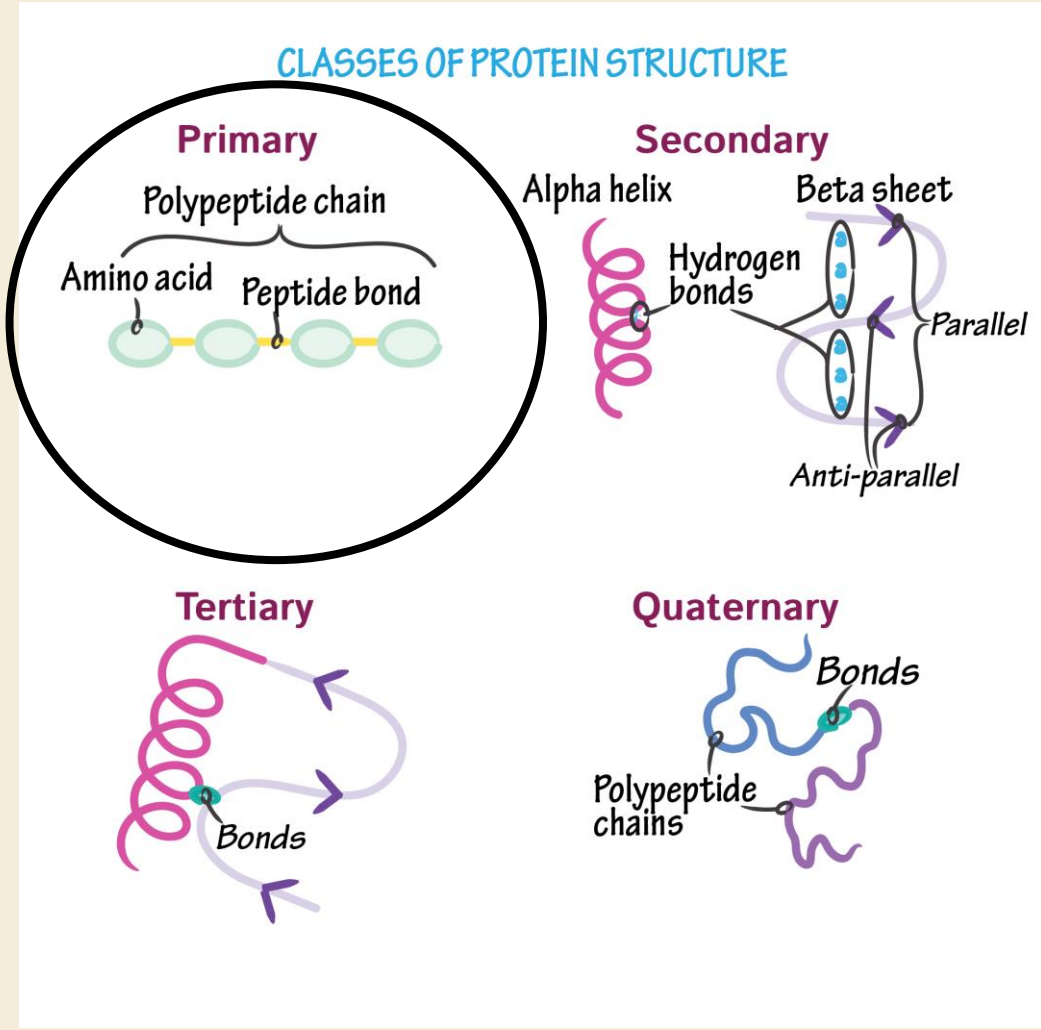


PRIMARY PROTEIN STRUCTURE

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.



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

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.

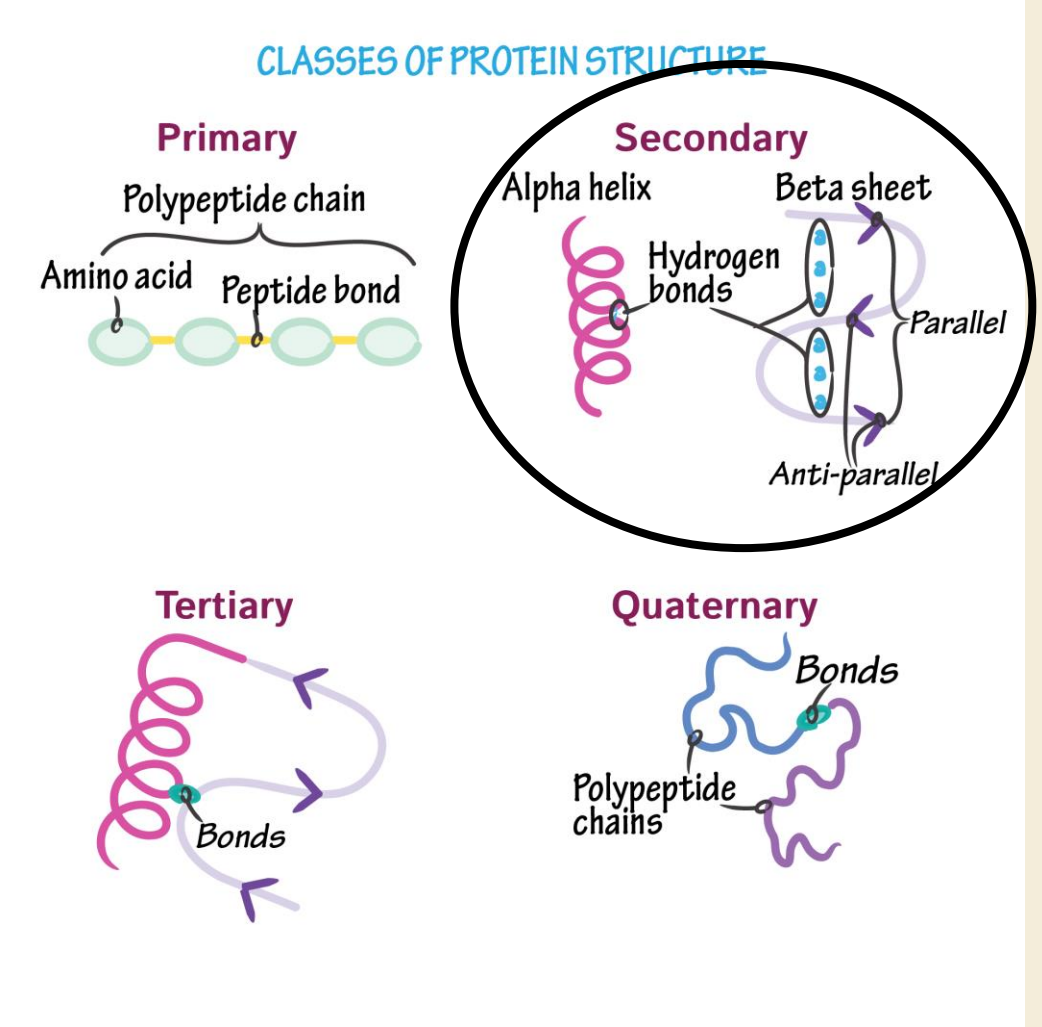
Alpha-helices

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.

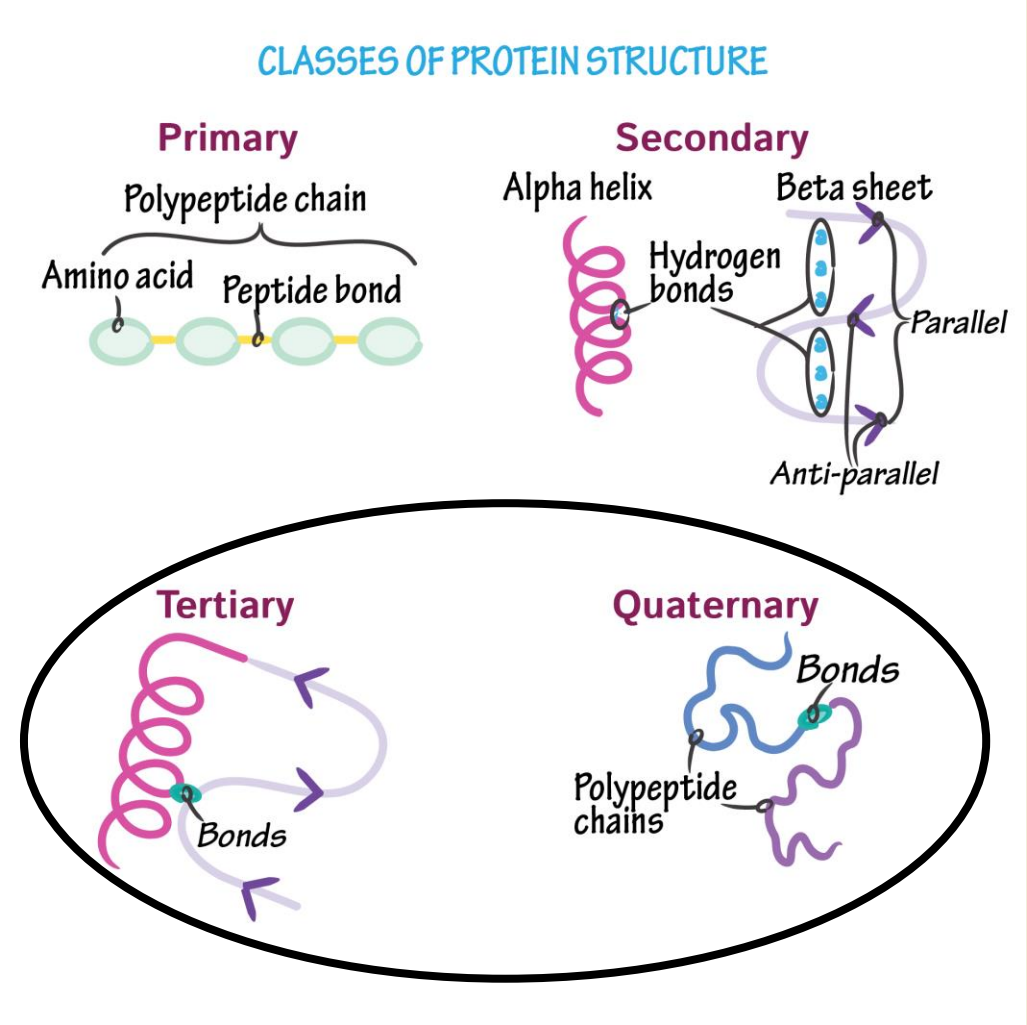
The following additional forces stabilize these structures:

-Hydrophilic interactions.

-Electrostatic interactions.

-Hydrogen bonds between side chains.

-Strong disulfide bonds.



Chemical bonds in proteins

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

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

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

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

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Electrostatic Interactions

A. Ionic Bonds (salt bridges)

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.

B. Hydrogen Bonds

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

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.

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

Hydrophobic Interactions

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 an interior, hydrophobic, protein core, where most hydrophobic sidechains can closely associate and are shielded from interactions with solvent .

Van der Waals Forces

The Van der Waals force is a transient, weak electrical attraction of one atom for another. Van der Waals attractions exist 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 sheer number (in big number). Most atoms of a protein are packed sufficiently close to others to be involved in transient Van der Waals attractions. This can be seen in the case of the model peptide, embedded in the dense interior of the chymotrypsin protein 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.

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

By chemical forces we mean also 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 watery fluids of an organism, within the blood, tissues, or within a cell itself.

A ligand can be natural, as an organic or inorganic molecule. Some ligands can be made synthetically, in the laboratory because the key properties of a ligand are found in its chemical structure → drug synthesis.

Ligands are typically used in cellular signaling and cellular regulation.

How LIGANDS work and which is their function

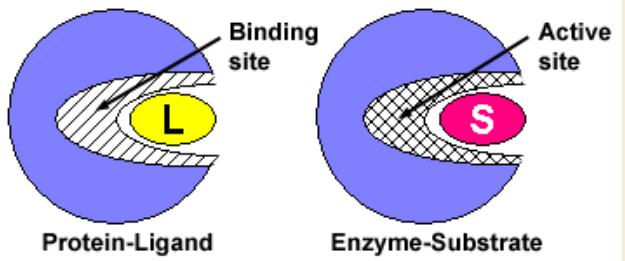
The ligand binds to the protein by weak interactions, inducing a conformational change of the protein. The physical action of the ligand changes the overall shape of the entire structure without breaking or forming new chemical bonds.

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.

The reversibility of the bond between ligand and protein is a crucial aspect of all forms of life. If ligands bound irreversibly, they could not serve as messengers, and most biological processes would fall apart. 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 ligands 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. The amino acids will match the ligand in certain aspects. For instance, both will be hydrophilic or hydrophobic. This increases the attraction between the substances. The amino acids tend to differ from the ligand in terms of electrical activity. If the ligand is positively charged, the binding site should be negatively charged. This creates the strongest interaction. In this way, proteins 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.

https://biologydictionary.net/ligand/

Examples of a Ligand

Oxygen

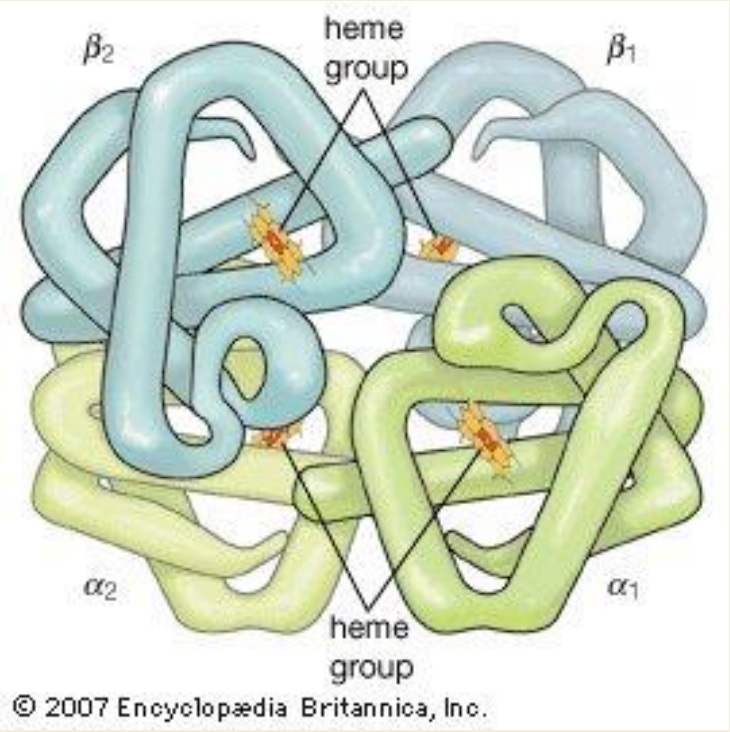
In the body tissues, oxygen must reach all the mitochondria in the body if the organism is to survive. But it is not an easy task to get oxygen everywhere. All organisms of a certain size must contain some sort of circulatory system and use specialized proteins for this.

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 red blood cells squeezing in capillaries. Oxygen diffuses then in tissue to reach the cells. RBC can then pickup carbon dioxide and bring it to lungs.

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

500 x 10^9 RBC / mL blood; 0.3 mL oxygen / 1 mL blood

Hemoglobin is a protein made up of four polypeptide chains (α1, α2, β1, and β2). 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.

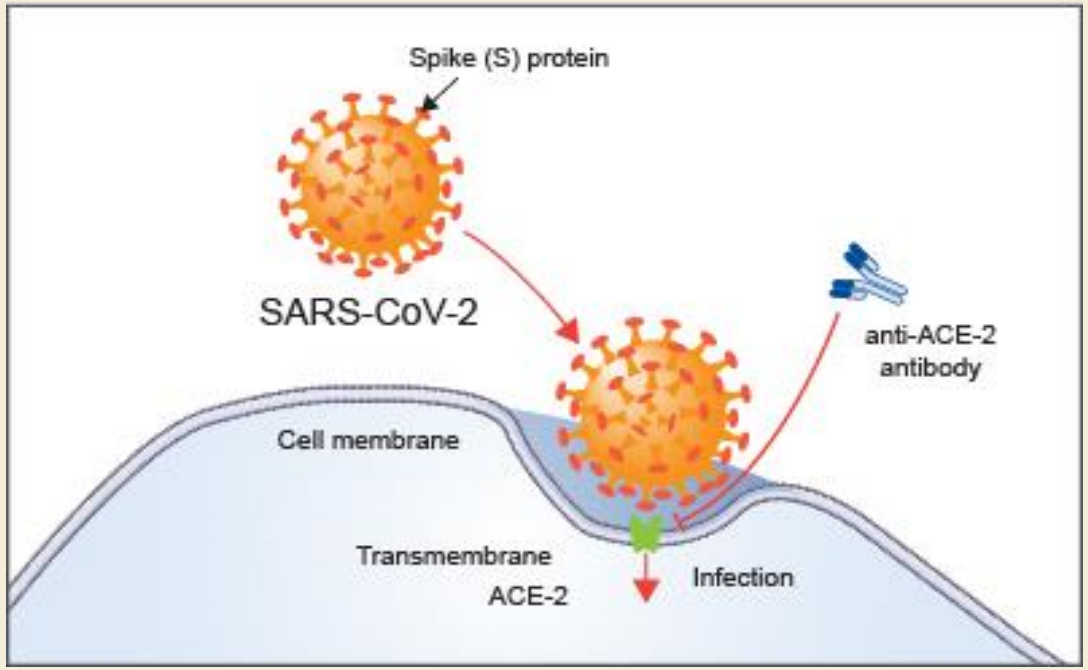
Dopamine

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.

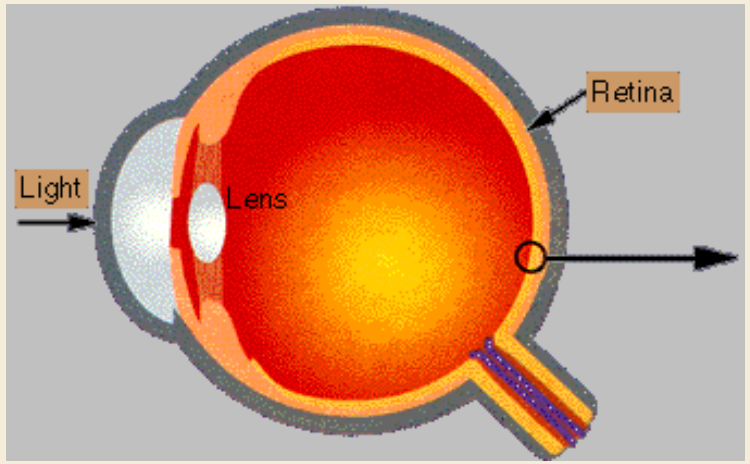
Bacteria and virus proteins

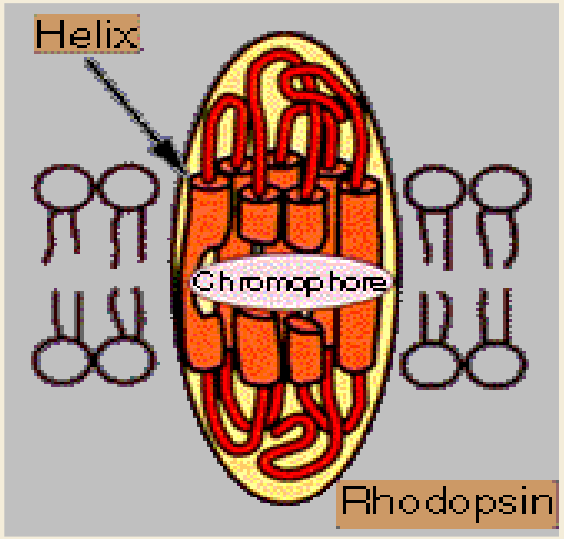
ACE-2 is the host cell receptor responsible for mediating infection by SARS-CoV-2, the novel coronavirus responsible for coronavirus disease 2019 (COVID-19). Treatment with anti-ACE-2 antibodies disrupts the interaction between virus and receptor.

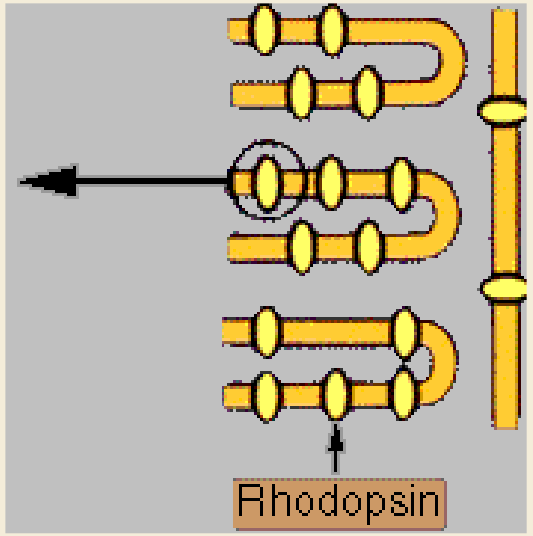


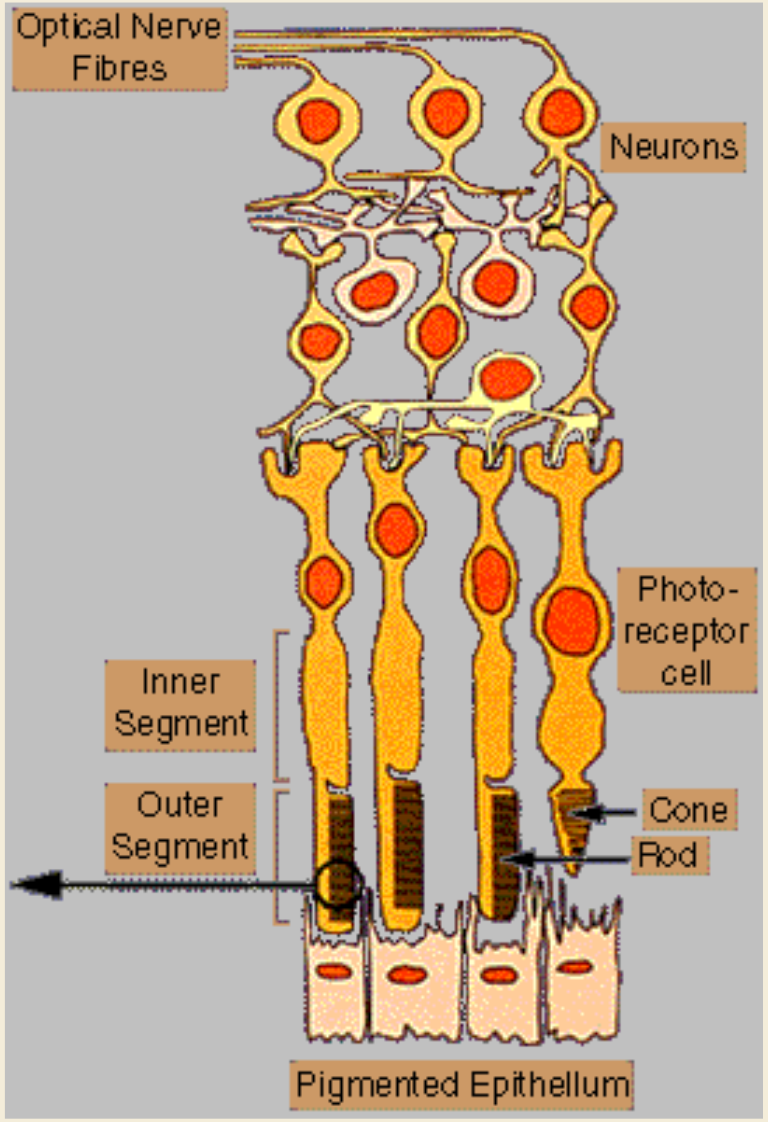
https://www.rndsystems.com/resources/articles/ace-2-sars-receptor-identified

The process of vision

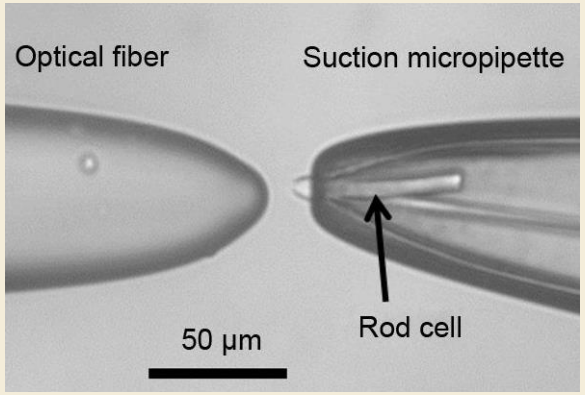








Lab exp.



http://nano-bio.ehu.es/files/seminar_retinal.pdf

Another example from phototransduction: **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. Rhodopsin 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

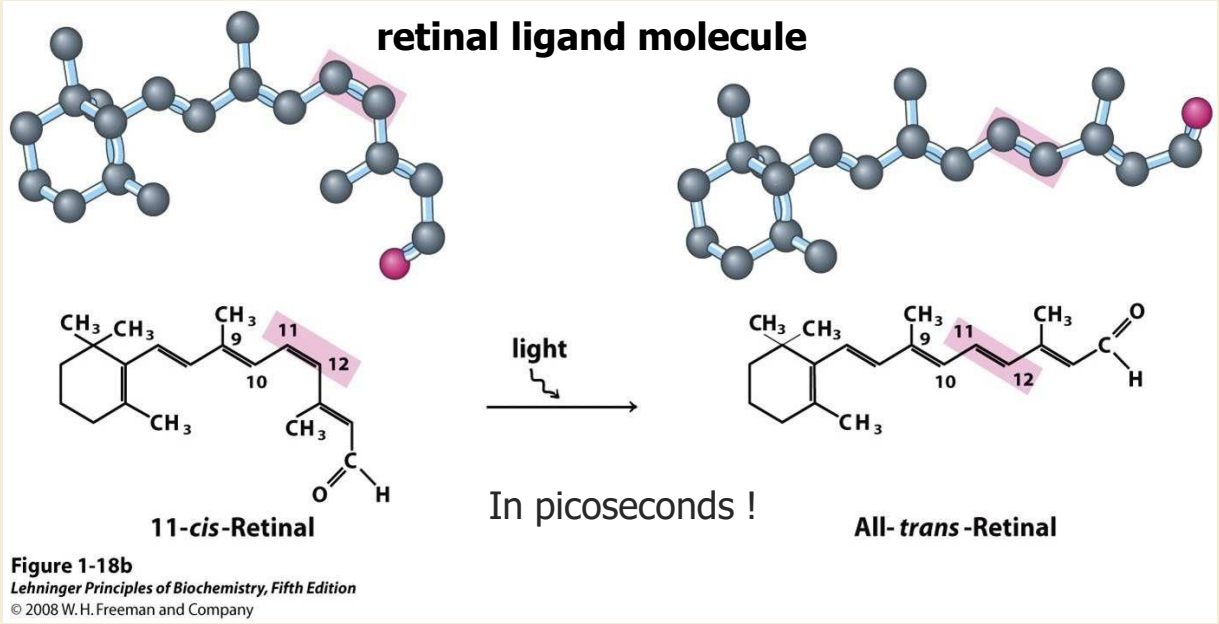
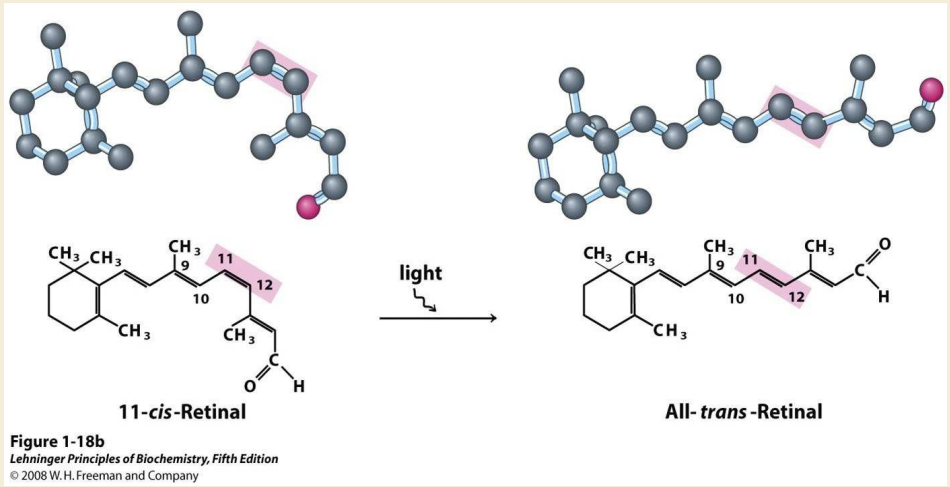


Figure 1-18b
Lehninger Principles of Biochemistry, Fifth Edition

http://nano-bio.ehu.es/files/seminar_retinal.pdf

Cis to trans isomerization

Isomerization process in vision: irradiation of Rh 11-cis-retinal isomerizes to all-trans-retinal : in appr. 200 fs, the photo-Rh is produced, a bathocromically shifted photo-product, which has a highly distorted trans conformation. This thermally relaxes to batho-Rh. Thermal relaxation keeps going all-trans-retinal + opsin. This is colourless (in contrast to former oranged rhodopsin). The all-trans-retinal is reduced to retinol, esterified, isomerized to 11-cis-retinol and oxidized to 11-cis-retinal recombination with opsin, and back to the beginning.



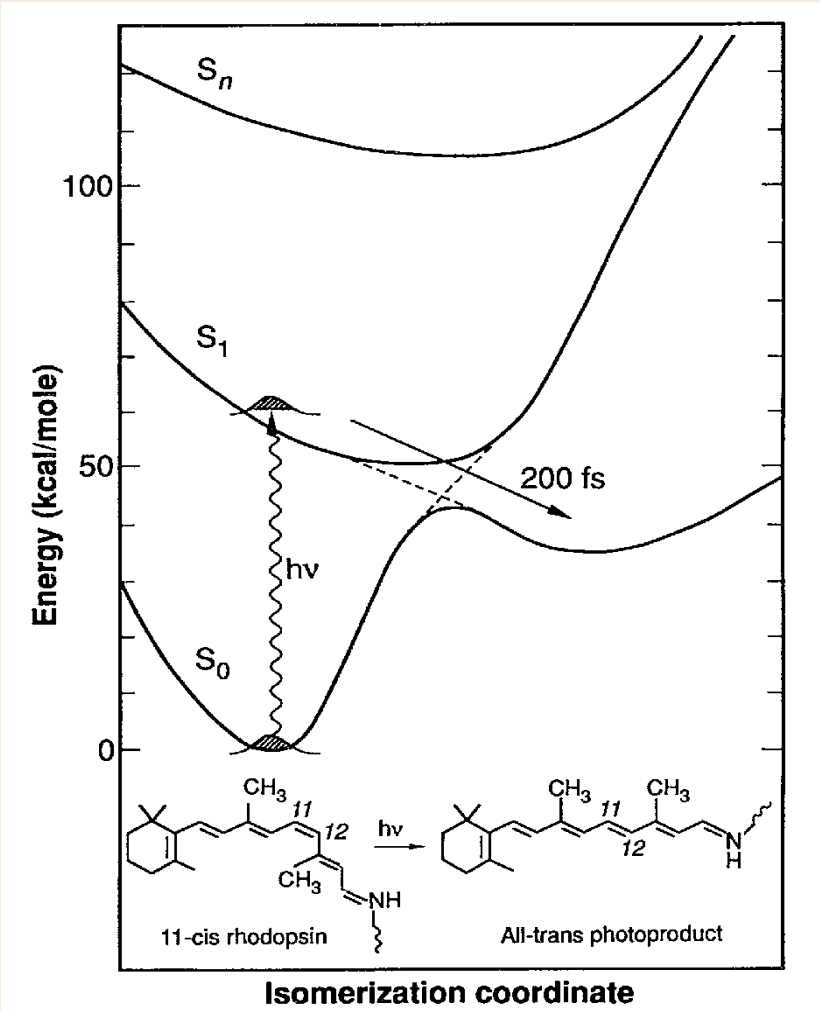
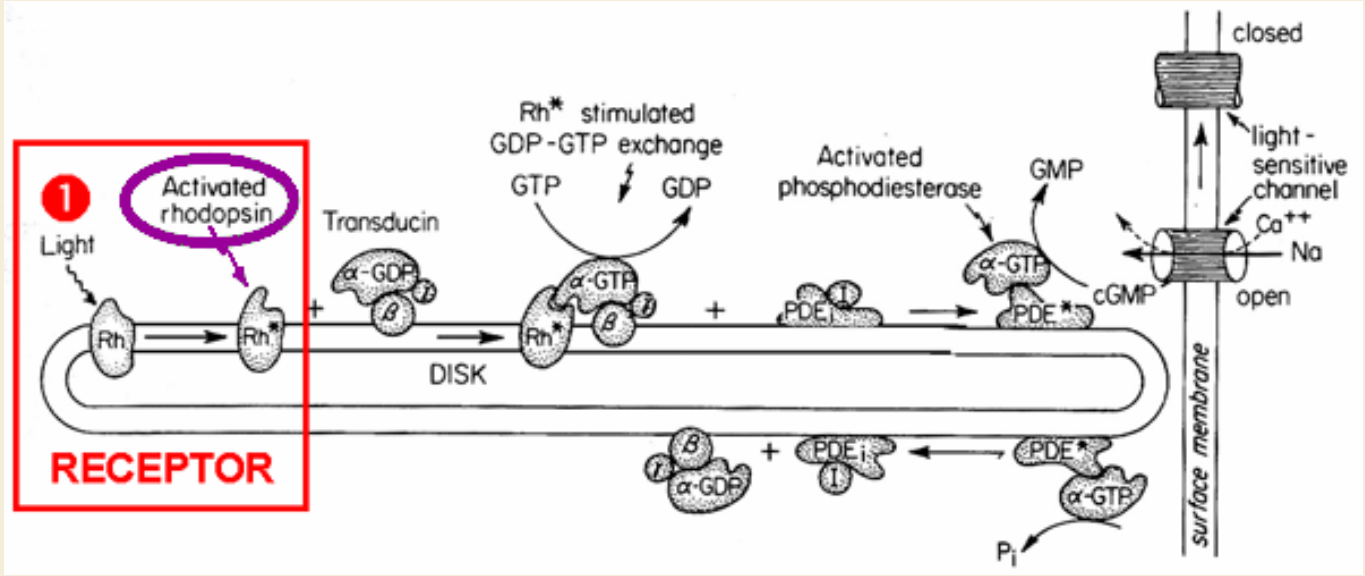


Fig. 1. Schematic ground-state and excited-state potential energy surfaces for the 11-*cis* → 11-*trans* isomerization in rhodopsin, adapted from (*14*). The reaction path of the photoisomerization is indicated by the nonadiabatic potential surfaces (broken lines).

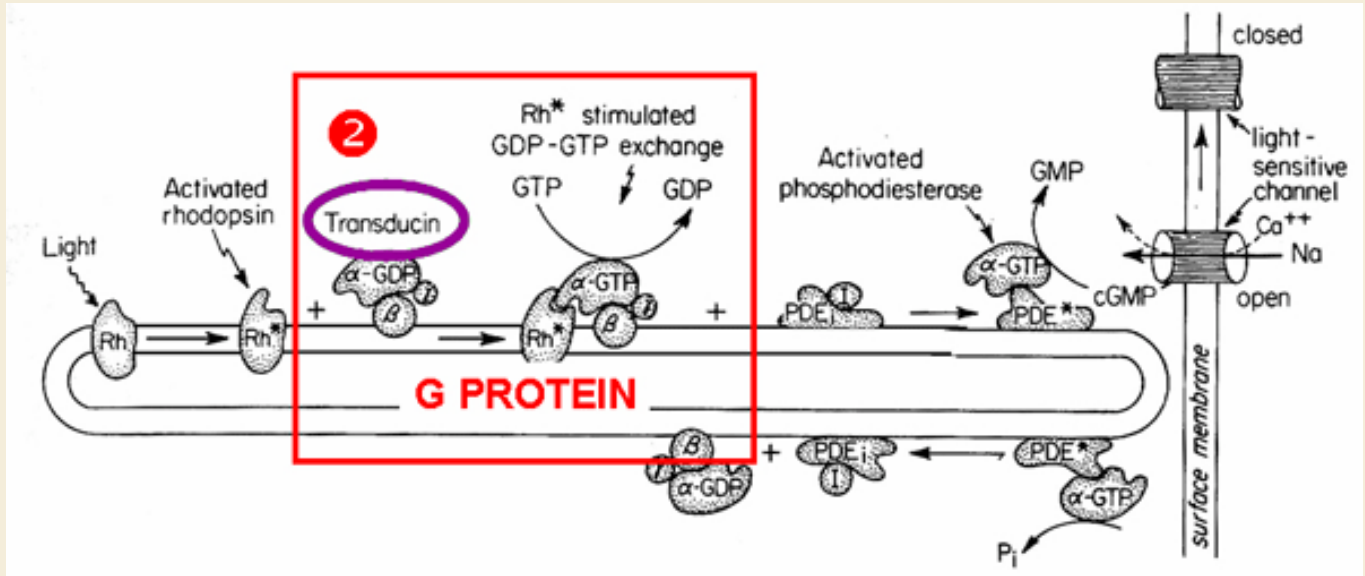
Schoenlein *et al*, Science **254**, 412 (1991)

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

1. activation of the receptor protein in rods, that is **rhodopsin**
   1 photon → 1 rhodopsin



2. the activated receptor protein stimulates the G-protein **transducin**
   GTP is converted to GDP in the process
   1 rhodopsin → 100 transducins/s

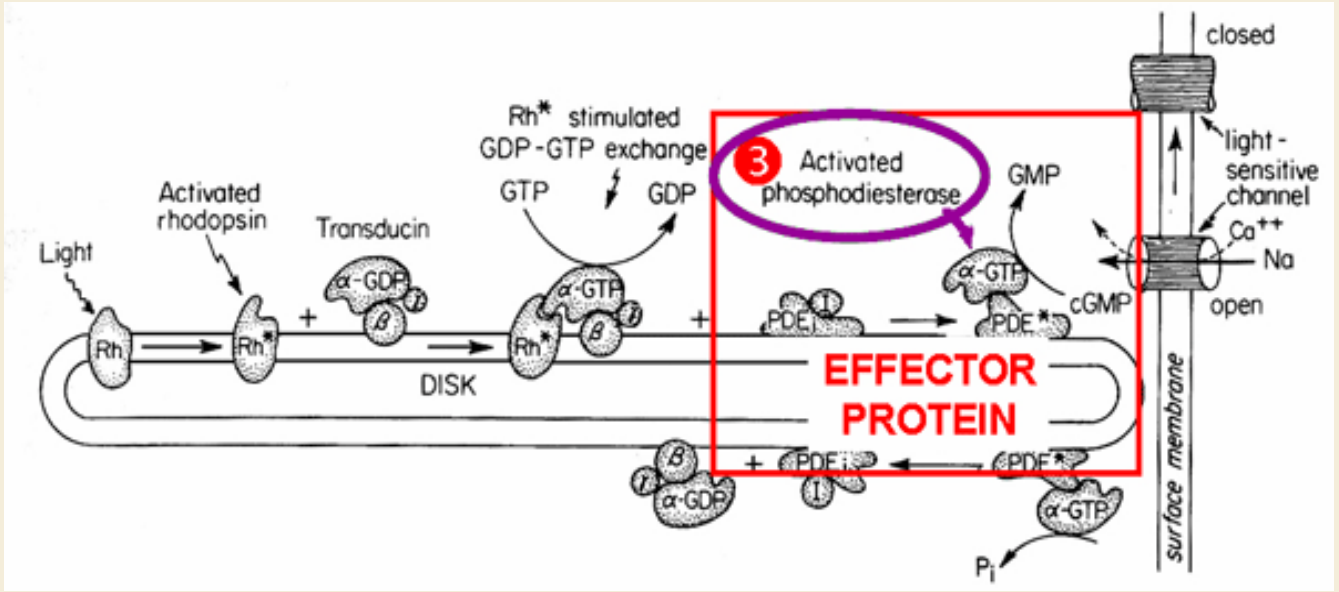


Transducin is a G (GDP) protein.
Once activated, GDP is exchanged for GTP from cytoplasm and alpha subunit dissociates

https://www.d.umn.edu/~jfitzake/Lectures/DMED/Vision/Retina/Phototransduction.html

3.In turn, activated transducin activates the effector protein **phosphodiesterase** PDE converts cGMP to GMP

1 transducin → 100 PDE/s

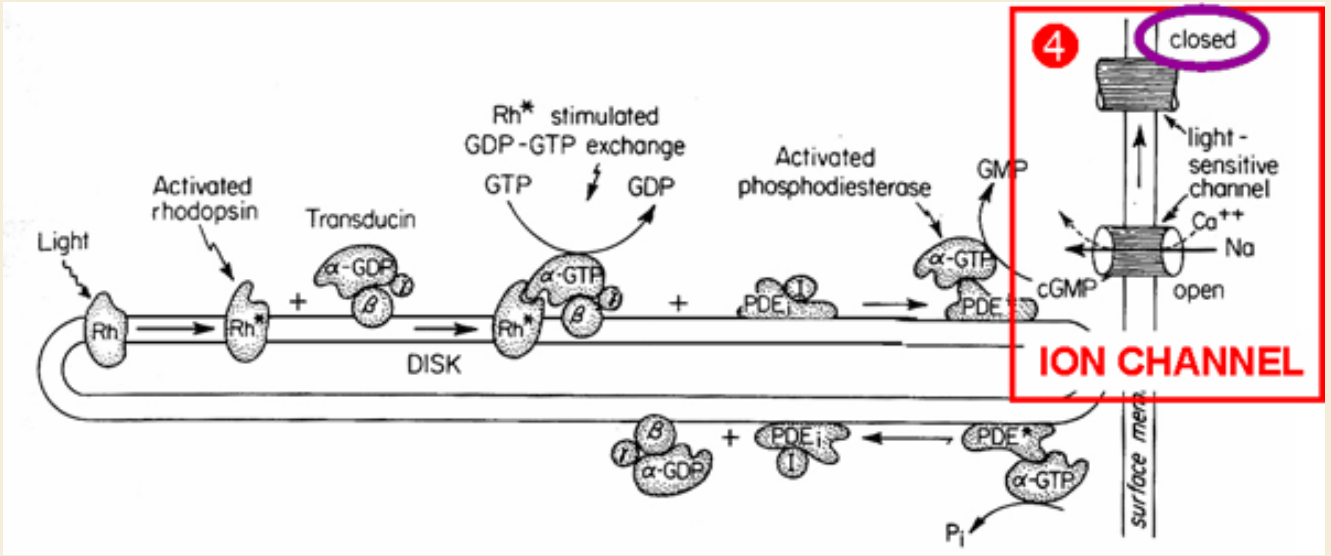


A phosphodiesterase (PDE) is an enzyme that breaks a phosphodiester bond in the second messenger molecule cGMP

Cyclic guanosine monophosphate (cGMP) is a cyclic nucleotide derived from guanosine triphosphate (GTP). cGMP acts as a second messenger. 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

4.Falling concentrations of cGMP cause the transduction channels to **CLOSE**, **DECREASING** a Na^+ current, resulting in a hyperpolarization of membrane potential

1 PDE → 1000 GMP/s



Amplification factor 10^7 !!! 1 photon → 1 rhodopsin → 100 x 100 x 1000

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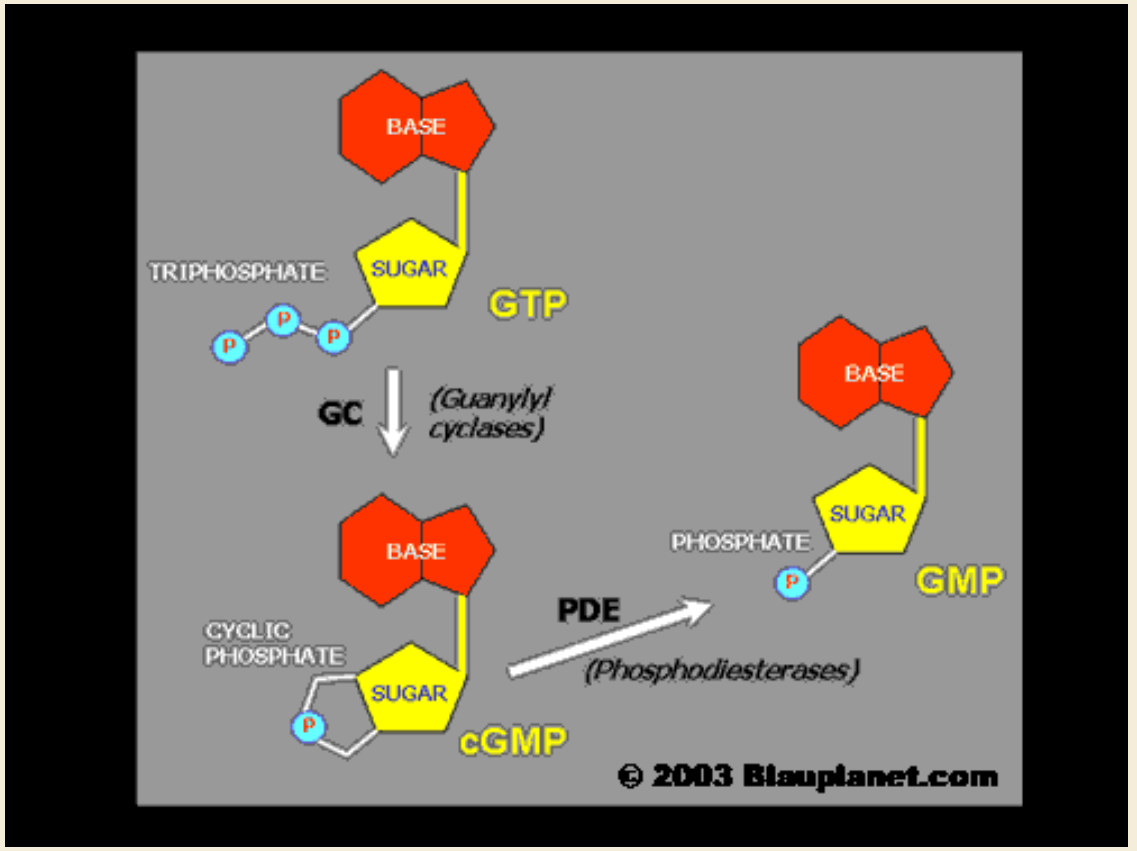
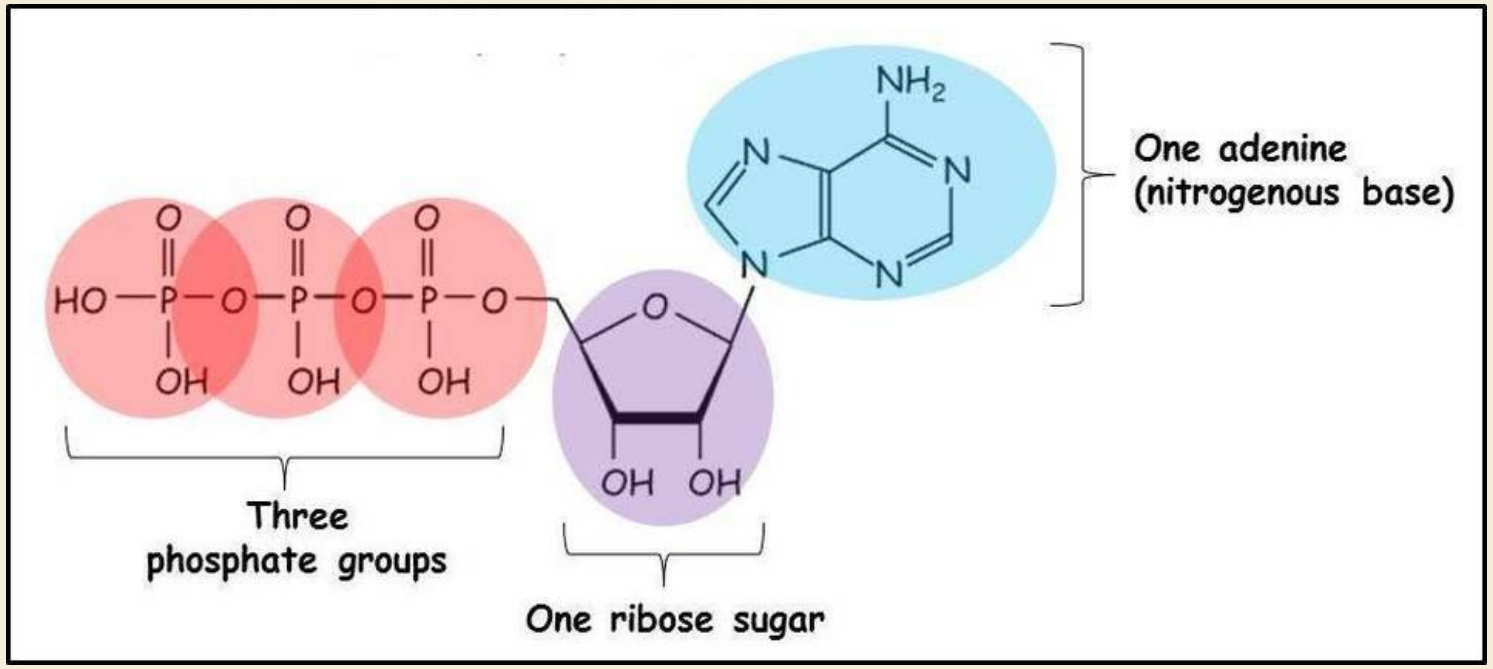
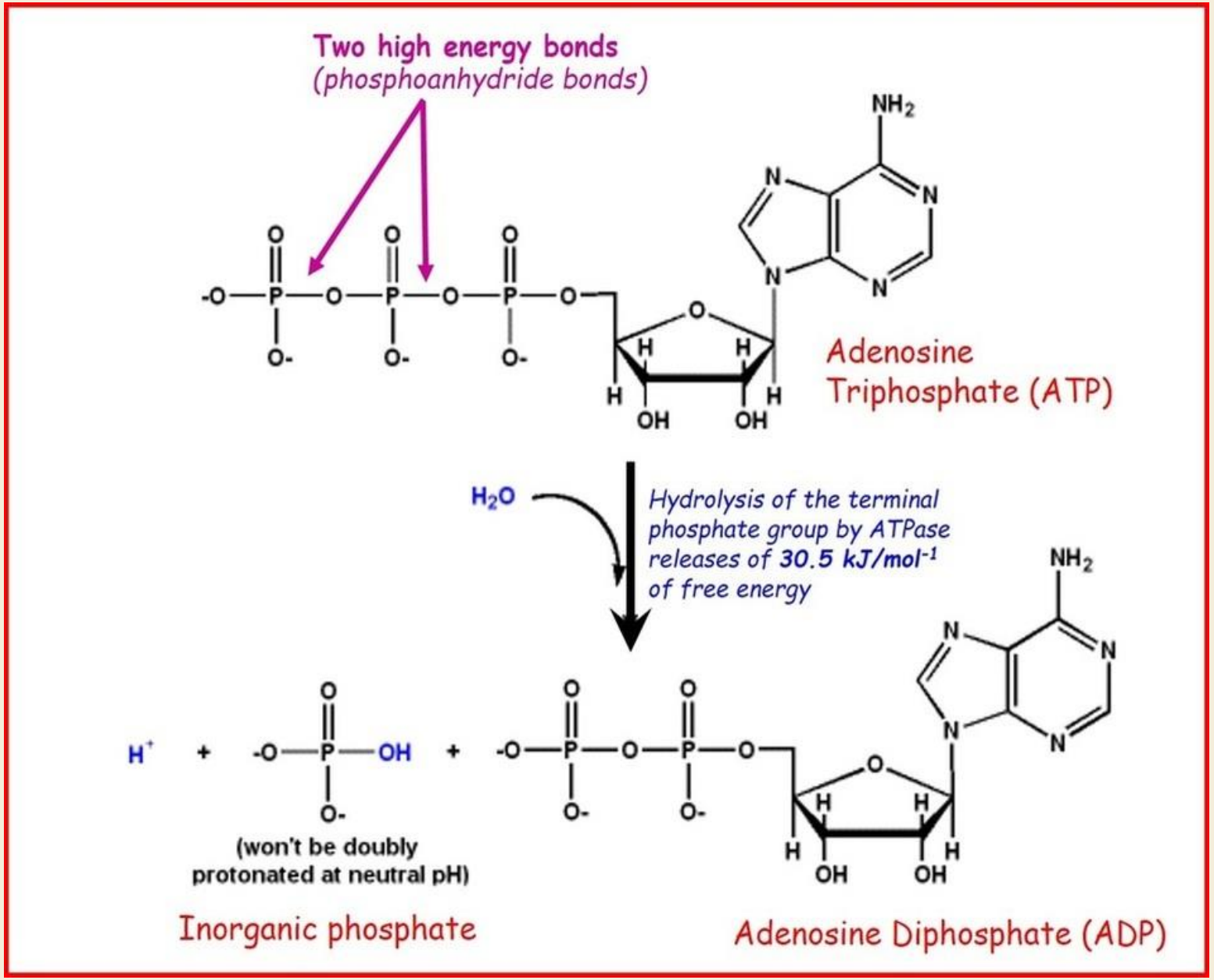


Another example – Adenosine triphosphate – ATP enzyme

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 + H20 → ADP + Pi + Free Energy

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 hydrolsed 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. A reaction that releases energy, such as ATP hydrolysis, is an **exergonic** reaction.

$$ATP + H_2O \xrightarrow[\text{hydrolysis}]{\text{ATPase}} ADP + Pi \qquad \Delta G = -30.5 \text{ kJ/mol}$$

$$ATP + 2H_2O \xrightarrow[\text{hydrolysis}]{\text{ATPase}} AMP + 2Pi \qquad \Delta G = -61 \text{ kJ/mol}$$

$$2ADP + 2H_2O \xrightarrow[\text{hydrolysis}]{\text{ATPase}} 2AMP + 2Pi \qquad \Delta G = -61 \text{ kJ/mol}$$

ΔG is the free energy change of the reaction. A negative value means energy is released

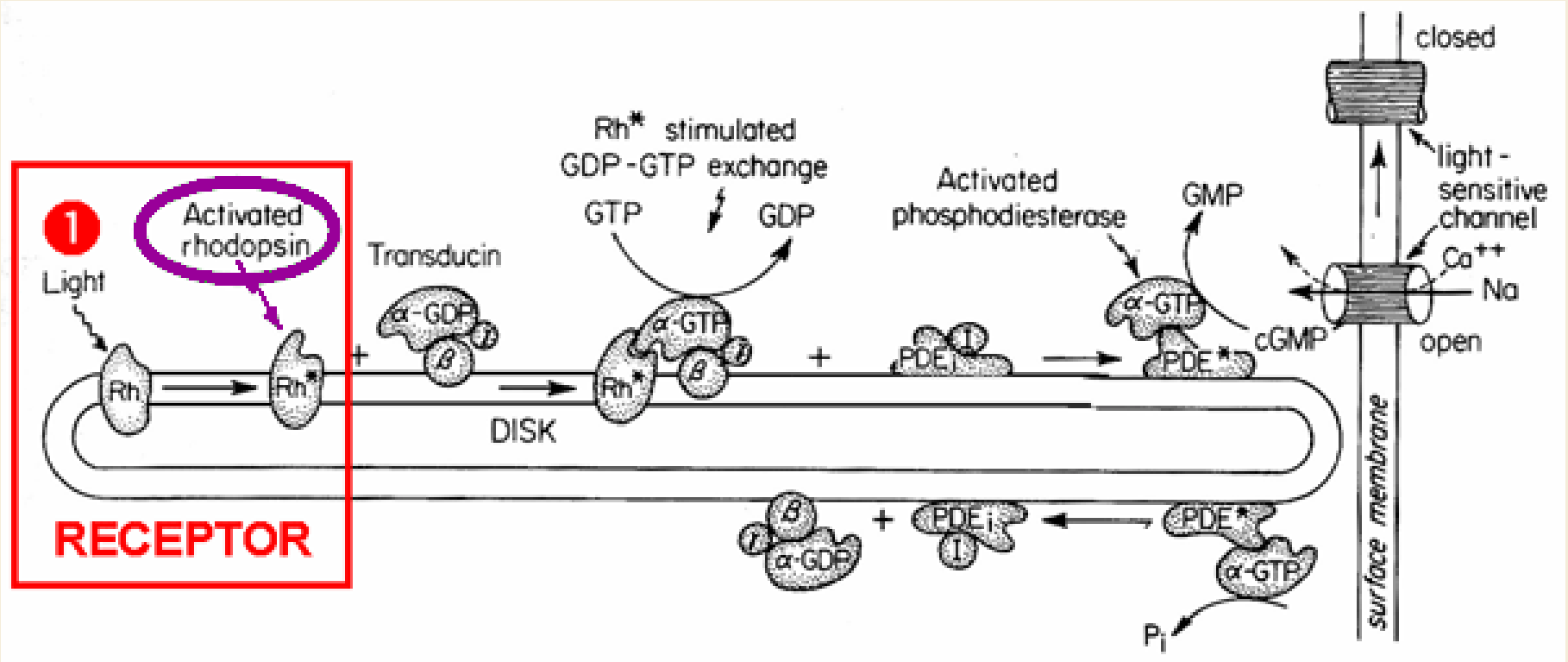
At pH 7 (e.g. inside cells):

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+$$

http://loretocollegebiology.weebly.com/atp-structure--function.html

Previous lesson we discussed:

- Chemical forces – intermolecular forces ("weak") types, energies
- Chemical bonds in protein, protein structure
- Ligands – examples (oxygen, retinal, ATP)



In the following, we will discuss how forces can influence chemical equilibria and the rate of 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 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 t**hat do not very too much from mean state (stable minimum energy state).

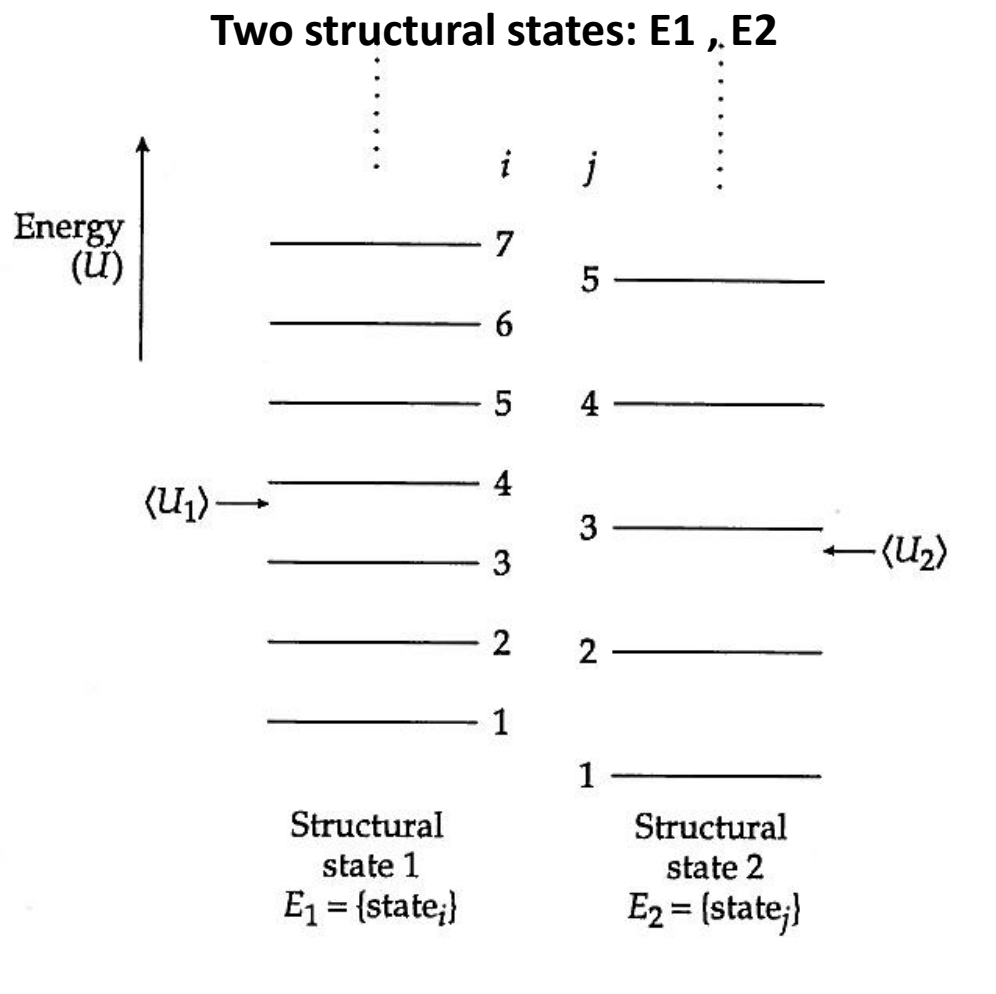
Due to the thermal fluctuations, a complex molecule like a protein can occupy an enormous number of different conformational states.
Conformational state: is defined by a set of coordinates of all the atoms.

Examples of different structural states:

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

Reference: Howard_Book_Ch 5 + papers cited in slides

The probability of finding the protein in one of the two (structural) states can be determined using the Boltzmann's law.



Ensembles of states

Suppose a molecule can be in one of two **structural states E1** and **E2.** Two different states have different reaction coordinates (e.g. lengths of the protein).

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

$$p_{i,j}=exp[-U_{i,j}/KT]$$

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

$$p(E1)= \sum p_i \qquad p(E2)= \sum p_j$$

This result is a generalized form of Boltzmann's law that relates the probabilities to the free energies.

(annex 5.1. of Howard's book

For a structural state we define the free energy as: ***G= <U> - TS***,

U comprises the internal energy – the energy associated with all the bonds (covalent, electrostatic, van der Waals) + other terms corresponding to the potential energies arising from external variables such as pressure, force, electrical fields or gravity.

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

The Boltzmann's law holds for ensembles of conformational states E1 and E2, if the energies are replaced by the Gibbs free energies, G1 and G2 (demo in annex 5.1. Howard):

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

$$\Delta G = G_2 - G_1$$

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

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 ration. Likewise, adding E2 pushes the reaction back toward E1.

Free Energy associated with ensembles of conformational states

(1) $\langle U \rangle = \sum_{i=1}^{N} U_i P_i$

The average energy, N - number of different conformational states of a molecule

U_i - potential energy for conf. state i

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

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

annex 5.1. Howard

(4) The free energy $G = \underline{\langle U \rangle - TS} =$

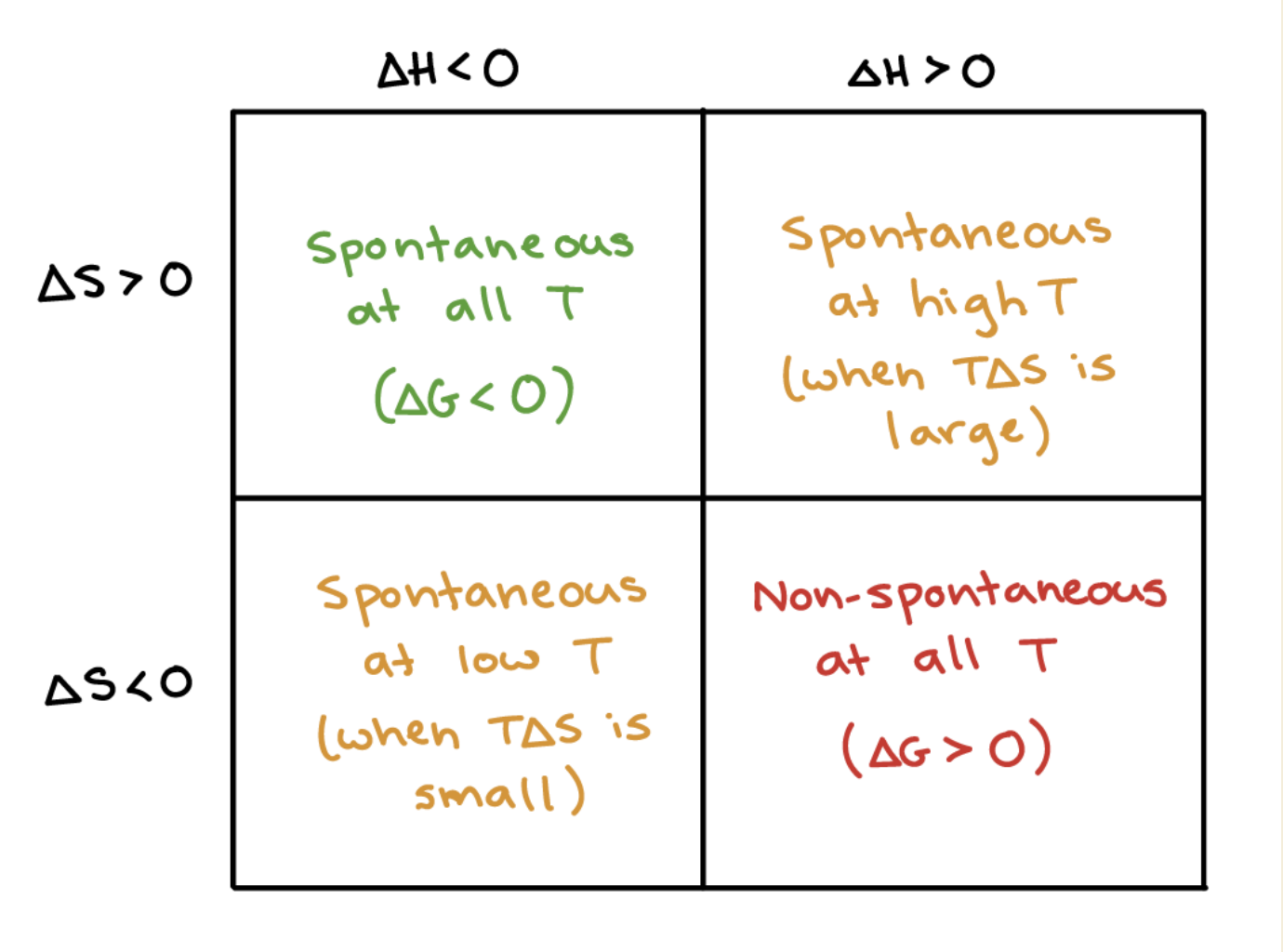
$$= \sum_{i=1}^{N} p_i (U_i + kT \ln p_i) =$$

$$= -kT \ln Z$$

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

In statistical thermodynamics free energy is expressed in terms of p_i - probabilities and energy levels U_i

$$[E_1] \to p_1 = \frac{z_1}{z} \quad [E_2] = \frac{z_2}{z} \quad \frac{[E_2]}{[E_1]} = \frac{p_2}{p_1} = \frac{z_2}{z_1} = \exp\left[-\frac{\Delta G}{kT}\right]$$



$G = \langle U \rangle - TS$

→

$\Delta G = \Delta H - T\Delta S$

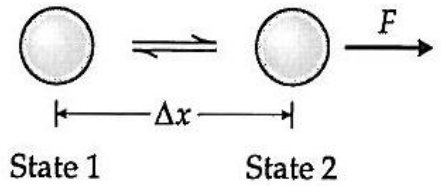
Force can influence the equilibrium between two (or more states).

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

Displacements associated with structural changes

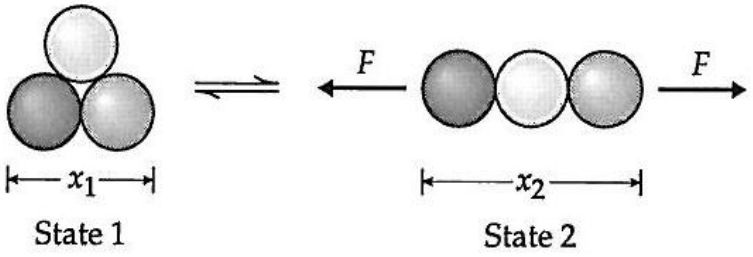
(A) Ex: motor moves along a filament

Translation

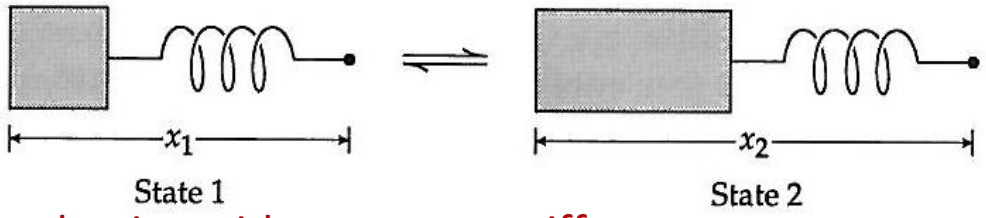


(B)

Lengthening



(C)



Lengthening with a constant stiffness

The difference in free energy G

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

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

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

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^0_{eq} 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.

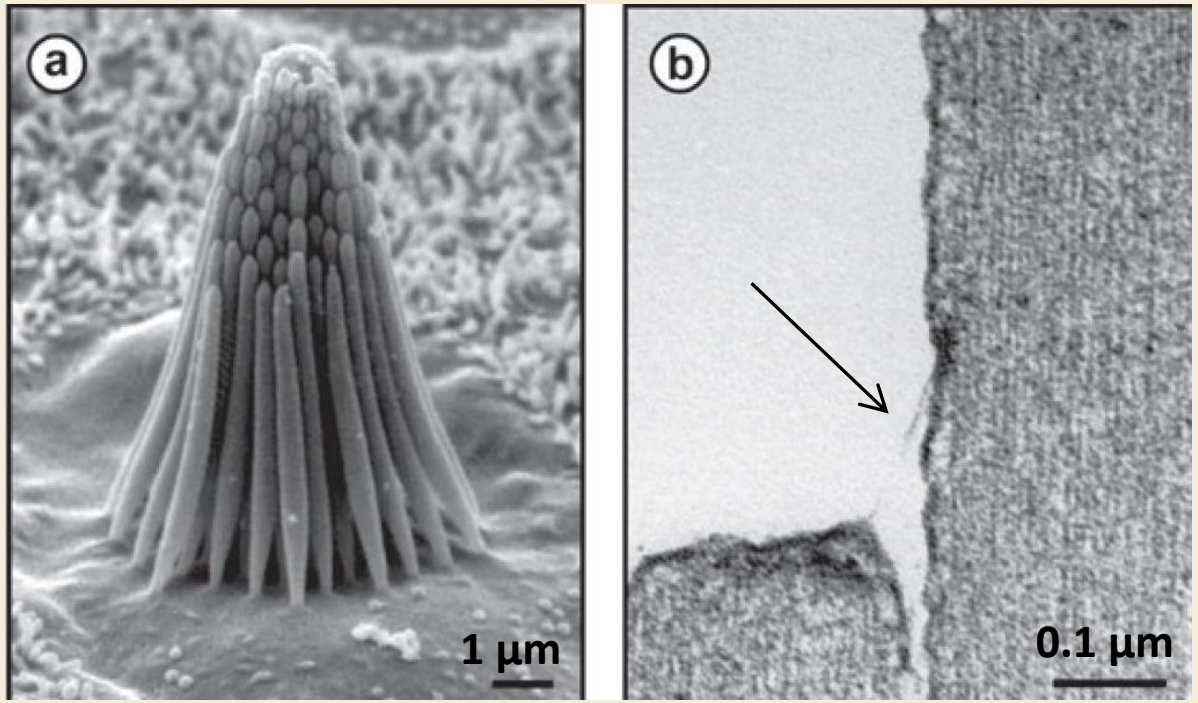
Example:

If the change Δx in the length of molecule is Δx = 4 nm, then a force F=1 pN will change the free energy by ΔG= 4 pN nm≈ 1 KT. **This will lead to an e-fold change in the ratio of concentrations** !

Protein conformational changes are measured in nanometers, and the energies range from 1 KT (thermal energy) to 25 KT (ATP hydrolysis)

→ **relevant biological forces will be on the scale of piconewtons**.

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



Hair cell anatomy:

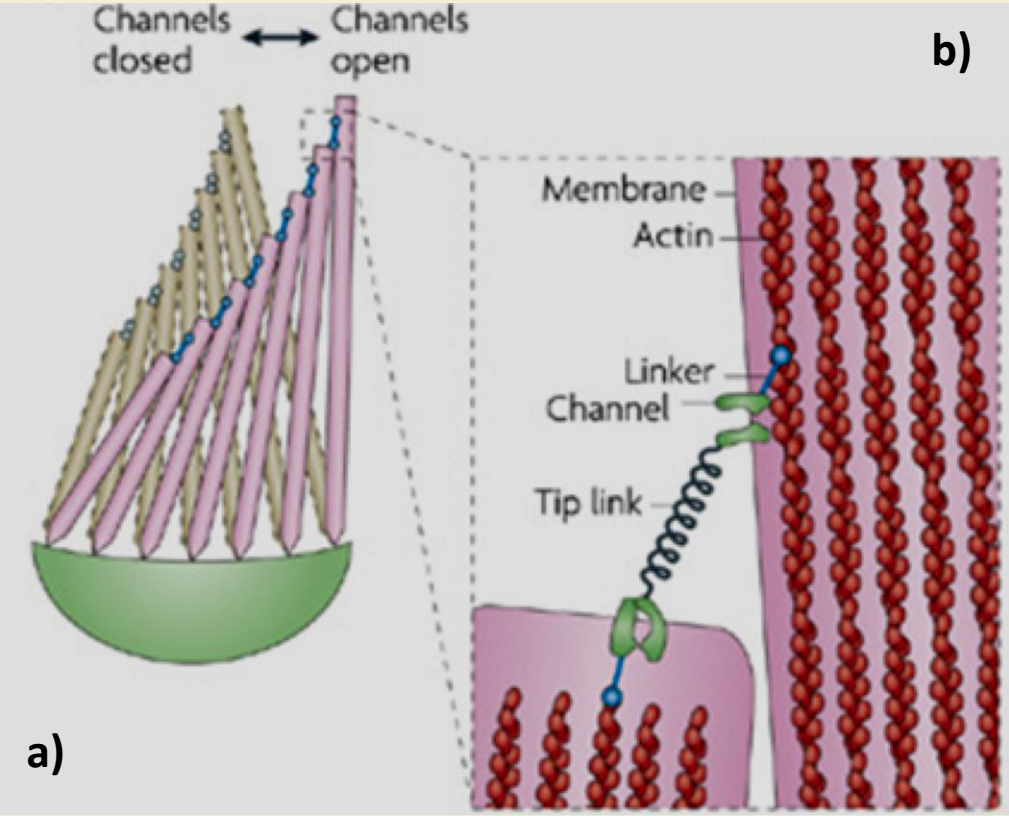
(*a*) Hair bundle in a bullfrog saccule, comprising ~60 stereocilia

(*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



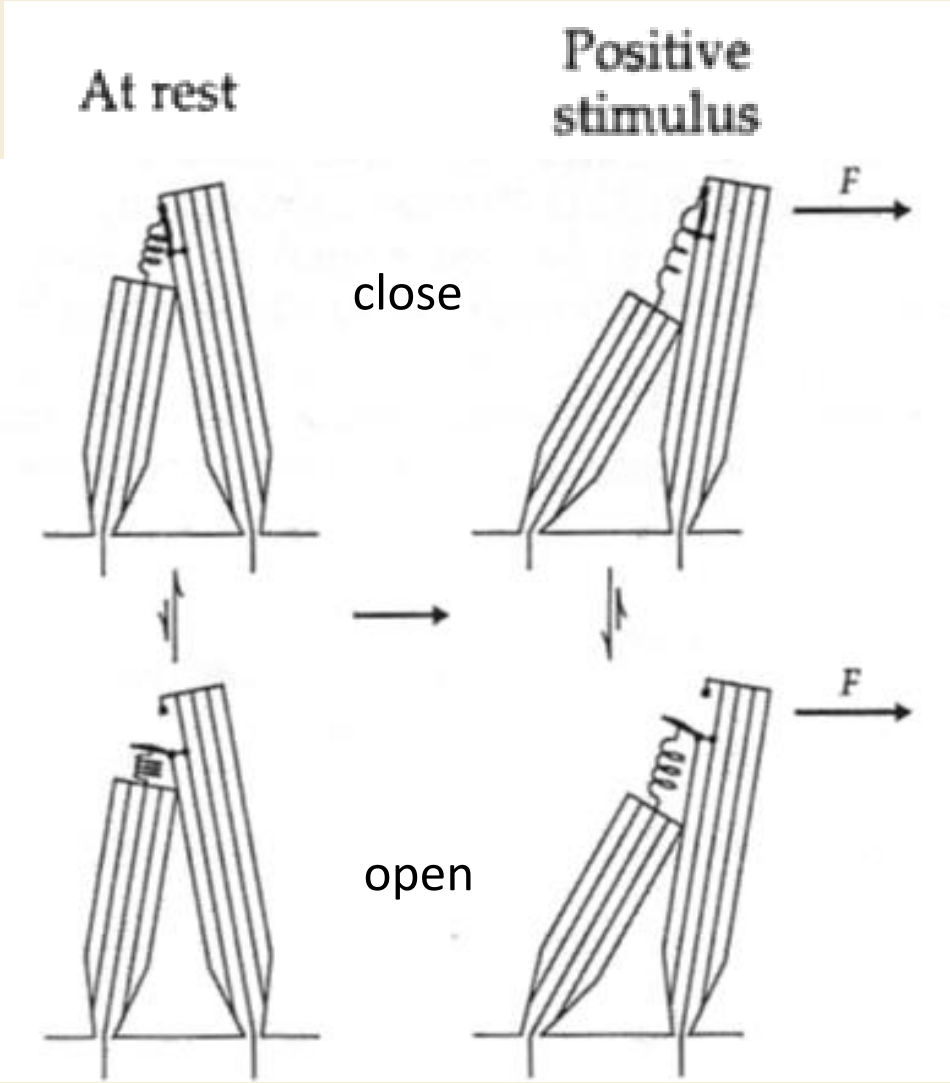
a) Hair bundle in resting (yellow) and deflected (pink) configurations.

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.

Hair-Cell Mechanotransduction Review and Cochlear Amplification

https://www.cell.com/neuron/pdf/S0896-6273(05)00881-0.pdf



External Force → deflection of hair bundle → shear between adjacent stereocilia → tension in the elastic tip link

→ pull and open ion channels.

The opening of a channel shortens the tip link

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

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

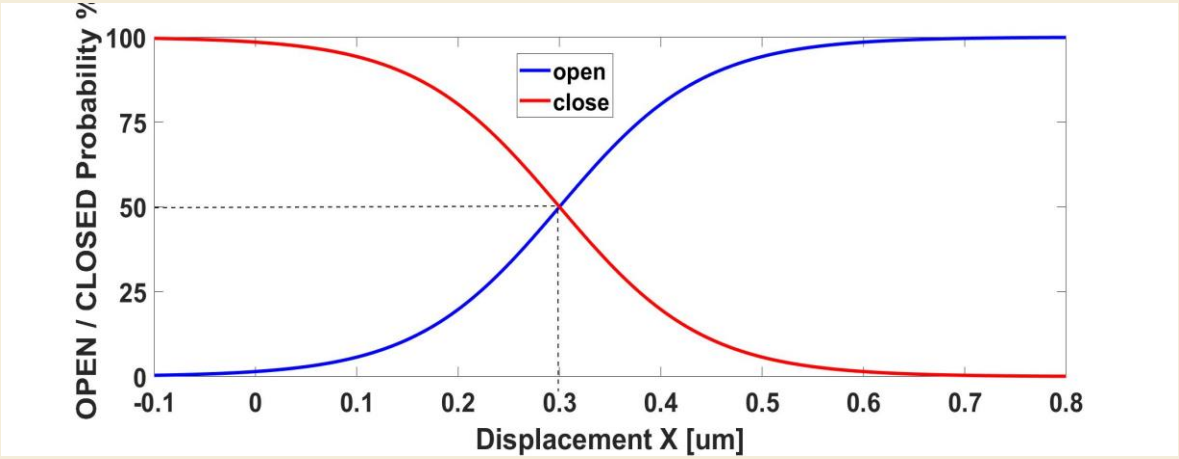
$$\frac{P_{open}}{P_{close}} = k_{eq}^{0} \cdot \exp\left(\frac{F\Delta x}{kT}\right) \qquad P_{open} + P_{close} = 1$$

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

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

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

Model



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

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

k - the stiffness of the tip link k= 2pN/nm

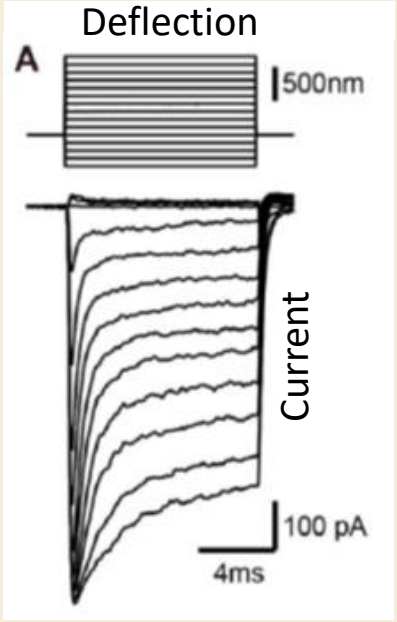
X – the displacement of the hair bundle (X=-0.1 – 1 um),

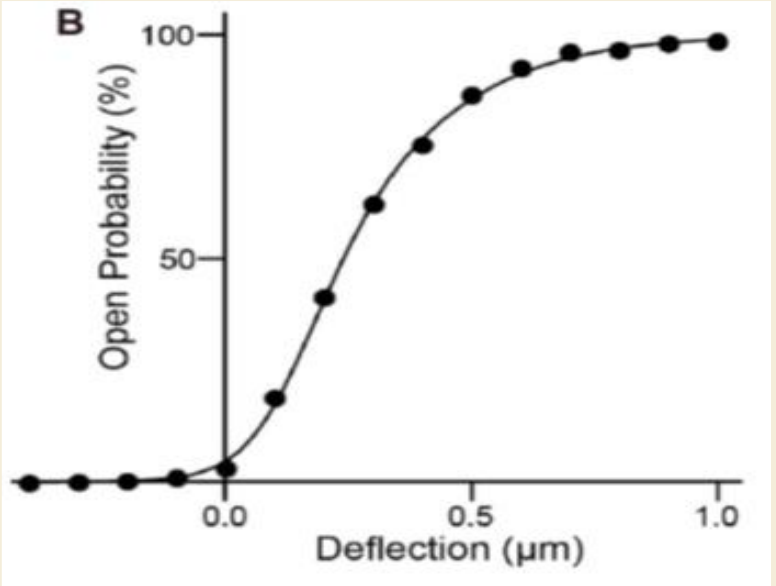
X0 – the displacement at which the channels are open 50% of the time, X0= 0.3 um; ***a*** - geometric factor (a>1)

Δx is the swing of the gate, Δx= 2 -4 nm, ***FΔx*** = *200 pN nm* ~ ***50 KT***

W th (a= 0.1, X-X0= 500 nm, Δx= 2 nm)~

Experiment





Forces also affect the rates of chemical reactions.

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

$$E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_2 \qquad \frac{d[E_1]}{dt} = -k_1[E_1] + k_{-1}[E_2]$$

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}].

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

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

This relation shows that the equilibrium constant $\boldsymbol{K_{eq}}$, defined with the concentrations, is equal to the ratio of the forward and reverse rate constants.

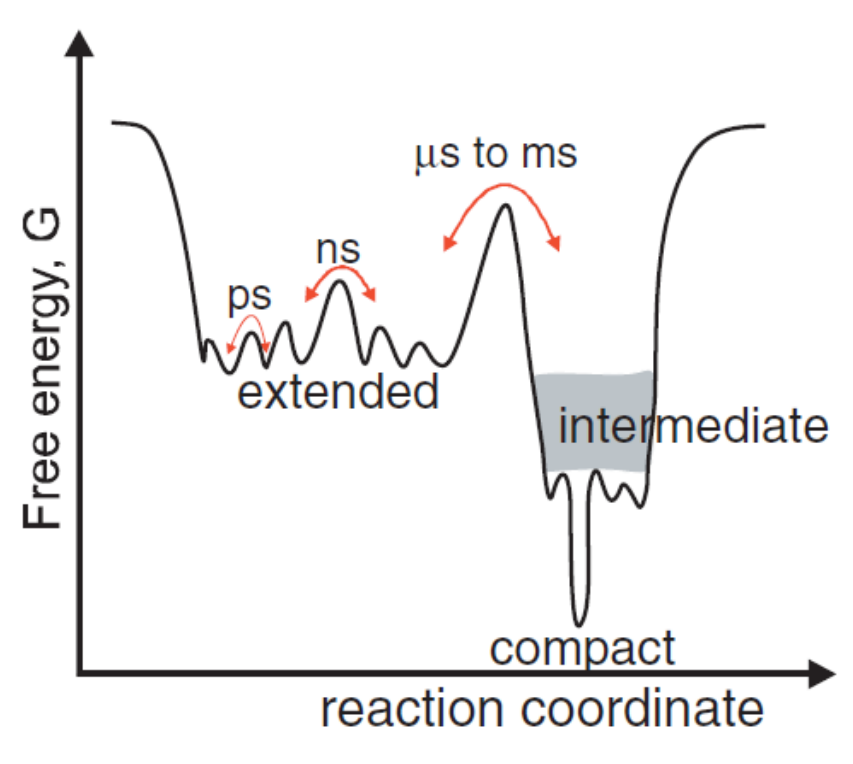
If the free energy difference between product and reactant ΔG depends on the force, then either the forward or the reverse rate (or both) must depend on force.

The transition between two strucutral 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 ?

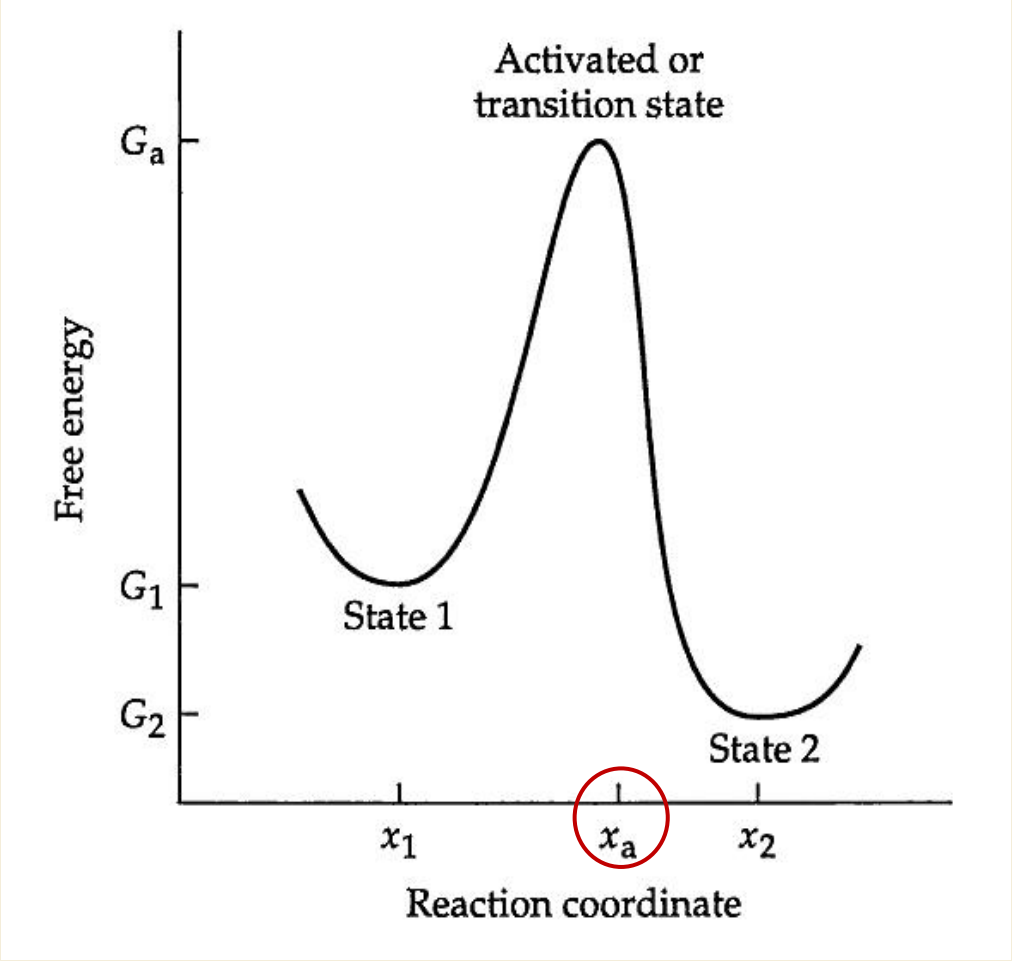
- Covalent chemical changes : about 0.1 picosecond;
- Global conformational changes: about 10 nanoseconds;
- Lifetime of the structural states : > 1 miliseconds .



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.

The activated-state concept leads to the idea that chemical reactions can be described using rate constants.

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.

$$k_1 = A\ exp\left[-\frac{\Delta G_{a1}}{KT}\right] \qquad \Delta Ga_1 = G_a - G_1$$

Arrhenius equation; *A*- frequency factor, or pre-exponential factor

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

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

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

$$\Delta G a_1 = G_{a1} - G_1$$

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 $E_1 \rightleftharpoons E_2$ 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, the distance corresponding to unbinding.

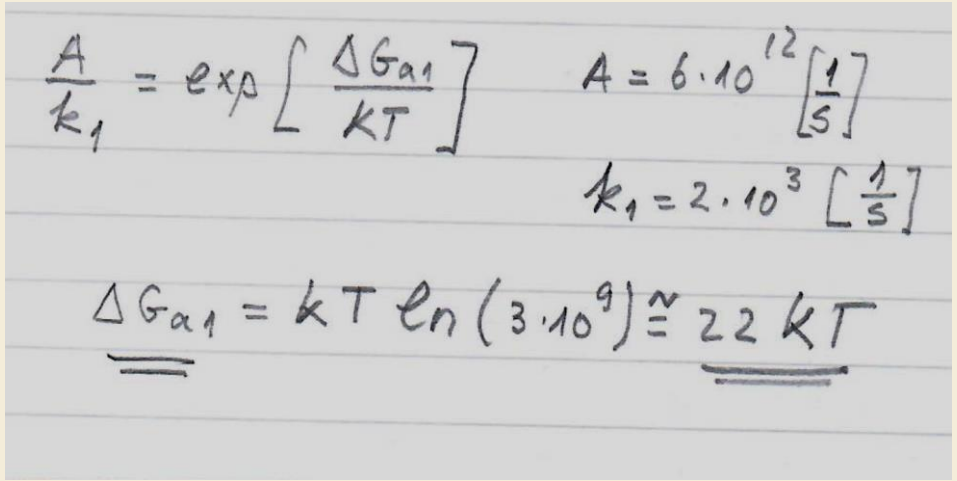
In the **Eyring rate theory,**

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

The frequency factor is ***A~ KT/h*** ≈ 6 x10^{12} [s^{-1}], where h is the Planck constant.

E.g. A reaction with a rate constant k_1= 2 x 10^3 s^{-1} , would have an activation energy : $\Delta Ga_1 = 22\ KT$

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



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.

$$k_1 = A\ exp\left[-\frac{\Delta G_{a1}}{KT}\right] \qquad \Delta Ga_1 = G_{a1} - G_1$$

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: $\boldsymbol{A \approx \frac{1}{\tau}\sqrt{\frac{\Delta Ga_1}{KT}}}$ with $\tau = \gamma/k$

The protein is sampling a different energy level every $\boldsymbol{\tau}$ seconds, because $\boldsymbol{\tau}$ 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 occuring is proportional to $exp\left[-\frac{\Delta Ga_1}{KT}\right]$.

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

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 $\tau = \gamma / k$.

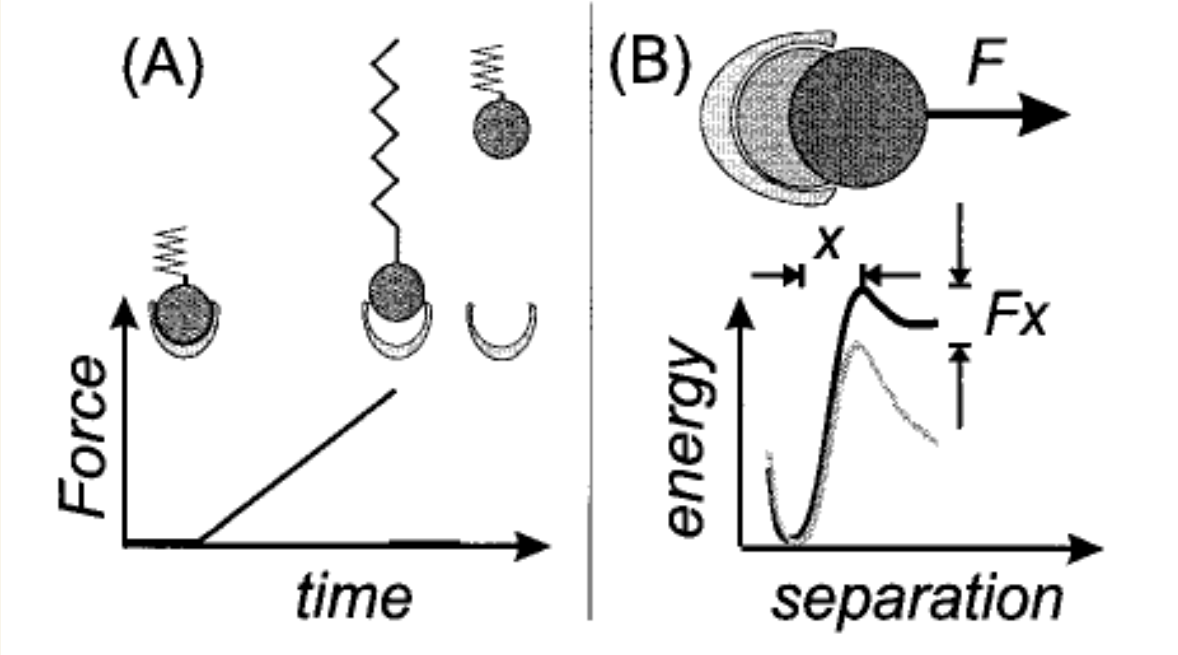
In the **Kramers** theory, the protein undergoes a global diffusion into the activated state.

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.

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.

(A) The dissociation over a sharp energy barrier is characterized by a linear decrease of the barrier with applied force *F*, giving rise to a characteristic length scale *x*.

T. Strunz et al, Model Energy Landscapes and the Force-Induced Dissociation of Ligand-Receptor Bonds, Biophys.J 79 (2000) - model

Eyring theory → Reaction rate without force

$$k_1^0 = \frac{KT}{h}\, exp\left[-\frac{\Delta G_{a1}}{KT}\right]$$

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

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

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

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

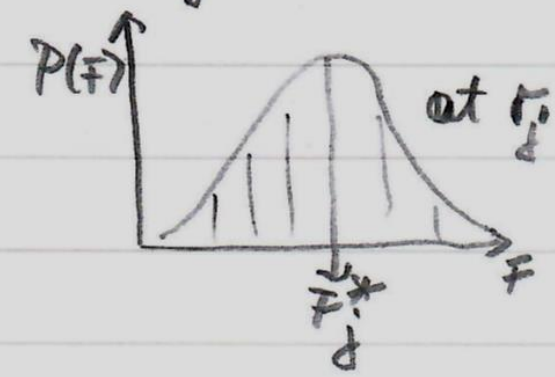
(6) The most probable unbinding/dissociation force

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

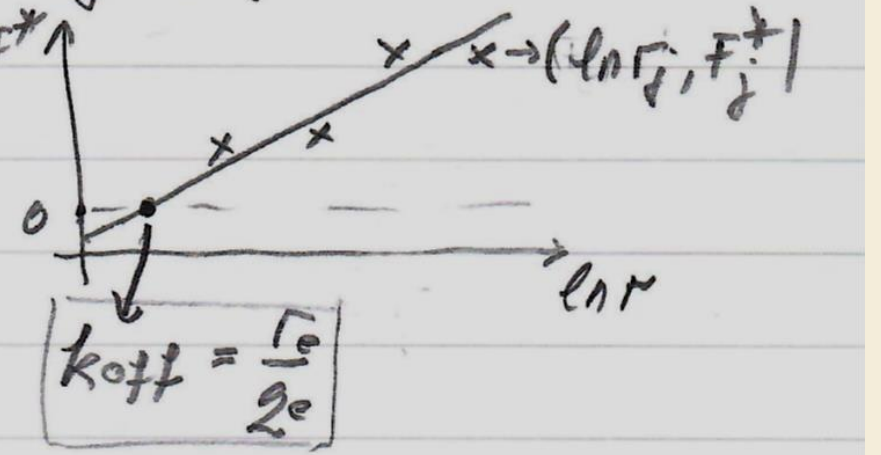
(7) $$F^* = 2\ln\left(\frac{r}{2\cdot k_{off}}\right) = 2\ln r - 2\ln(2\cdot k_{off})$$

F^* linear with $\ln r$

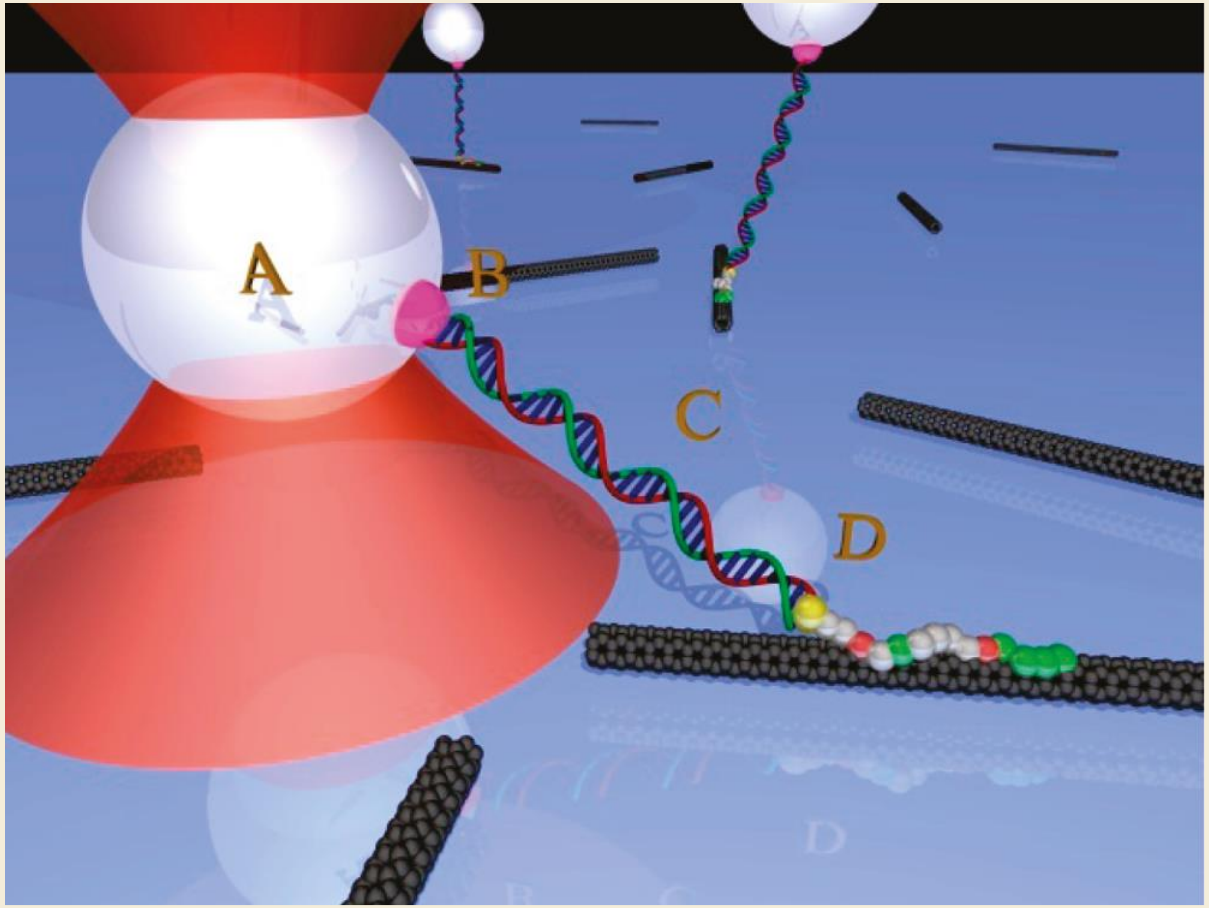
F_j^* measured for loading rate r_j, $j = 1 - N$ experiments



$j = 1 - N_{exp}$



$$k_{off} = \frac{r_e}{2^e}$$



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).

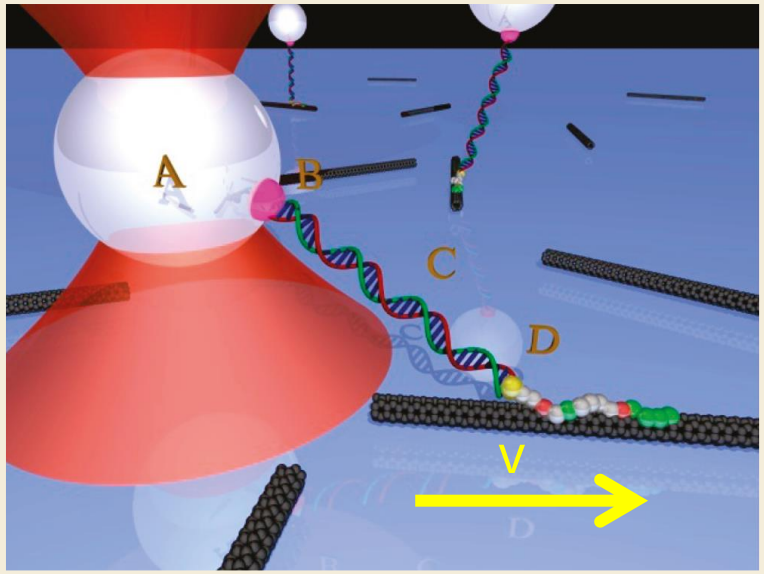
Adhesion through Single Peptide Aptamers

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

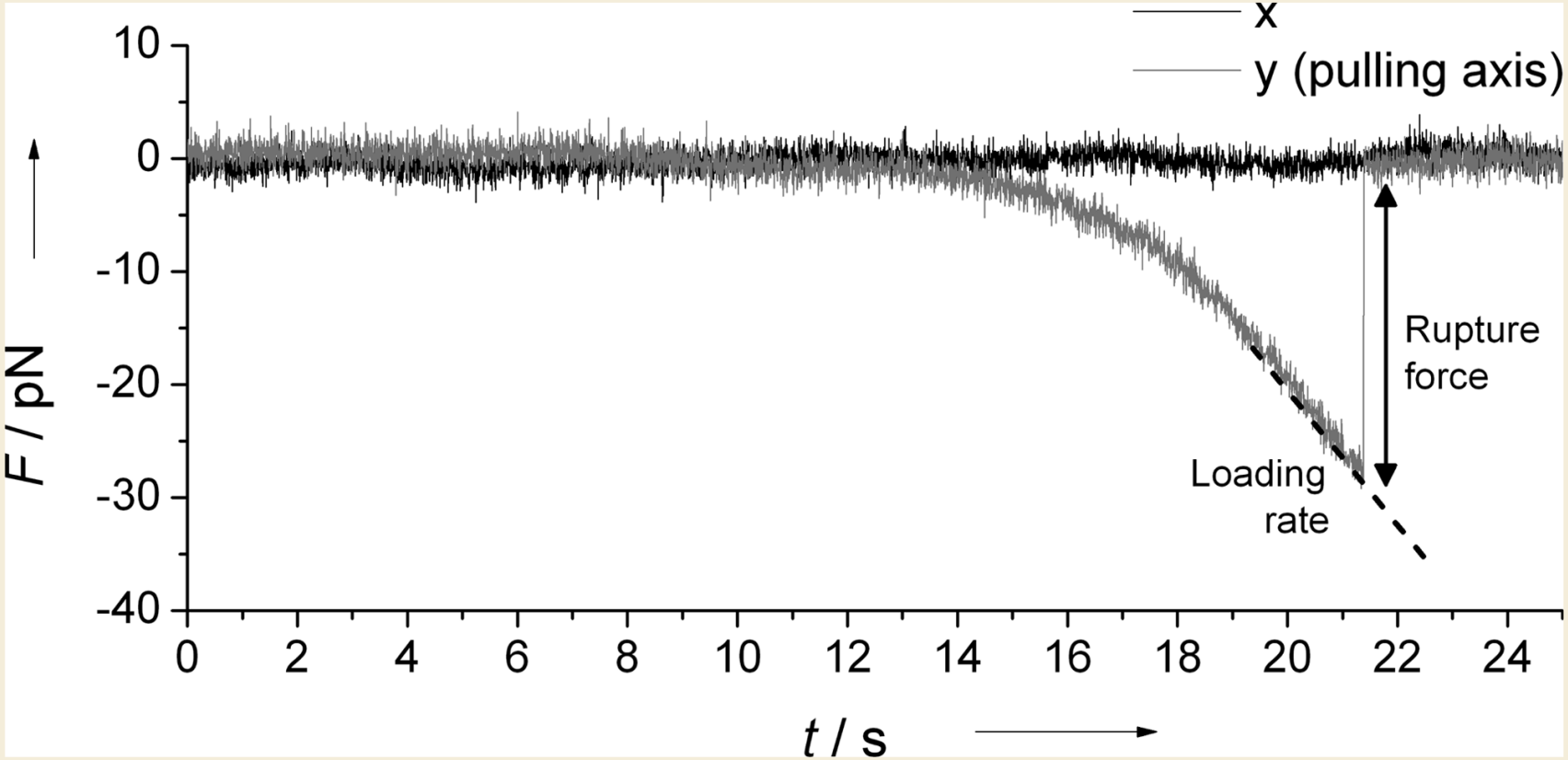
Peptide aptamers typically contain 8-20 amino acids and bind materials or biomolecules.

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

dx.doi.org/10.1021/jp1031493 |J. Phys. Chem. A 2011



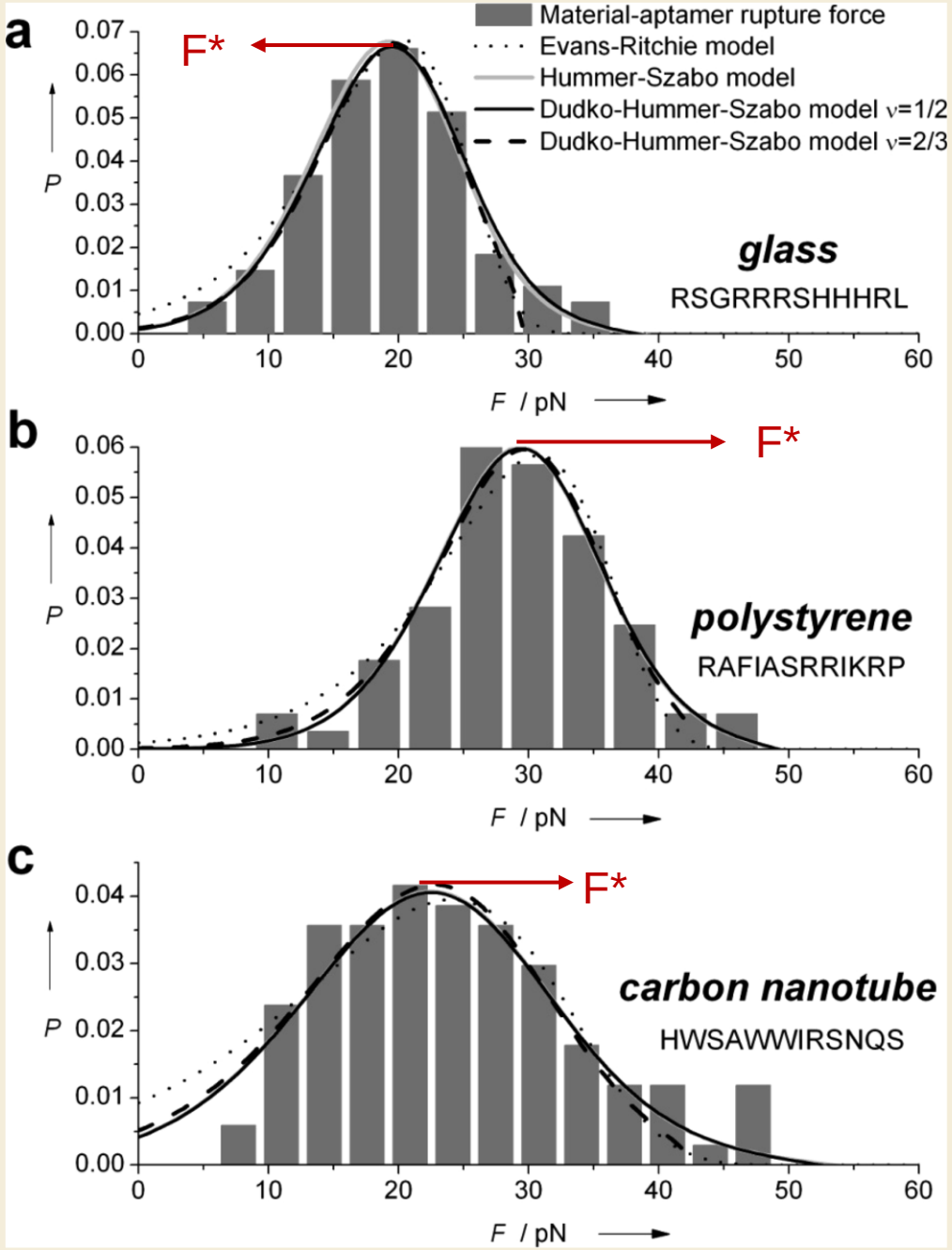
Rupture force measurement



Representative force curve showing a rupture event.

Rupture forces and loading rates, dashed line, are directly measured from rupture curves.

dx.doi.org/10.1021/jp1031493 |J. Phys. Chem. A 2011

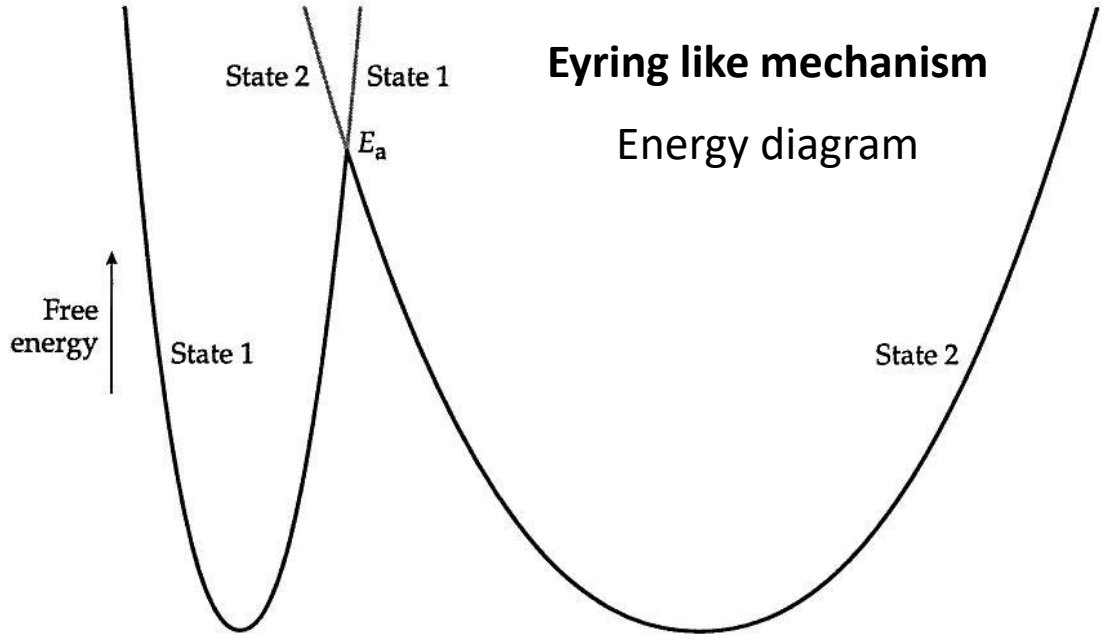


Rupture-force probability *P(F)* distributions for peptide aptamer binding to :

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

$\tau_0^a = {}^1/_{k_{off}}$	Evans–Ritchie model	
interaction	$\tau_0{}^a$	$x^{\ddagger\, b}$
glass/aptamer	96.9	0.747
polystyrene/aptamer	109.5	0.652
CNTs/aptamer	20.7	0.404

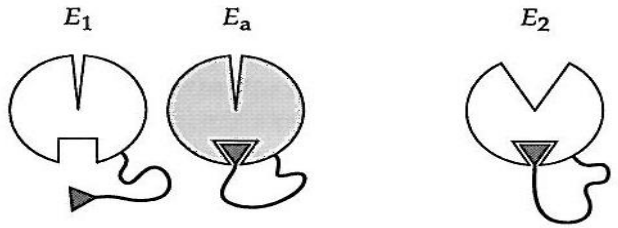
[a] Units are s. [b] Units are nm. [c] $\Delta G^{\ddagger}$ is in $k_B T$ units.



Eyring like mechanism

Energy diagram

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



Monomolecular model

The model of the working stroke of myosin.

After myosin has bound to actin in its pre-powerstroke state, the phosphate rapidly disocciates, leaving the protein in a highly strained post-powerstroke state.

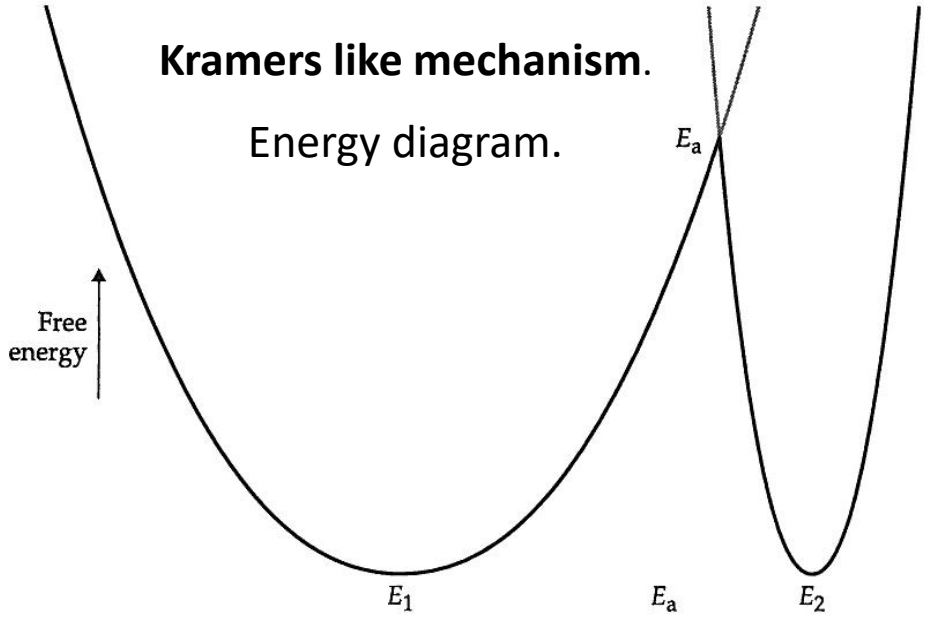
The relaxation of this highly strained state drives the sliding of the filaments and the shortening of the muscle. If the filaments are prevented from sliding, the strained state will maintain the tension in the muscle.

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 global conformation which then relaxes more slowly into a new stable state.

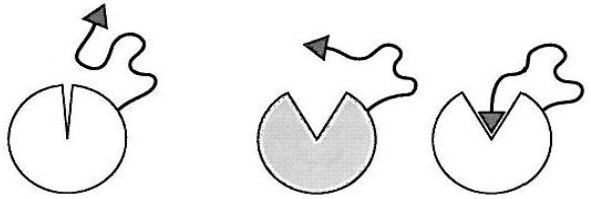
Kramers like mechanism.

Energy diagram.



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

Monomolecular model



Thermal ratchet model for motor proteins.

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

Consider a motor protein with k= 4 pN/nm and radius r= 4 nm. The free energy available from ATP hydrolysis is 25 KT. The drag coefficient ɣ= 60 pN s/m, and the relaxation time τ= 15 ns.

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

$$t_K = \tau\sqrt{\frac{\pi}{4}}\sqrt{\frac{kT}{U_0}}\exp\left(\frac{U_0}{kT}\right)$$

But for myosin, the complete ATP hydrolysis reaction only takes about 0.05s. 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, a more reasonable efficiency is 50 %, and the corresponding pick up time becomes only t_k= 7 ms. The kinetics of myosin becomes thus consistent with Kramers like mechanism.

More details on the mechanisms in force generation in motor proteins – 2. 3 Motor proteins

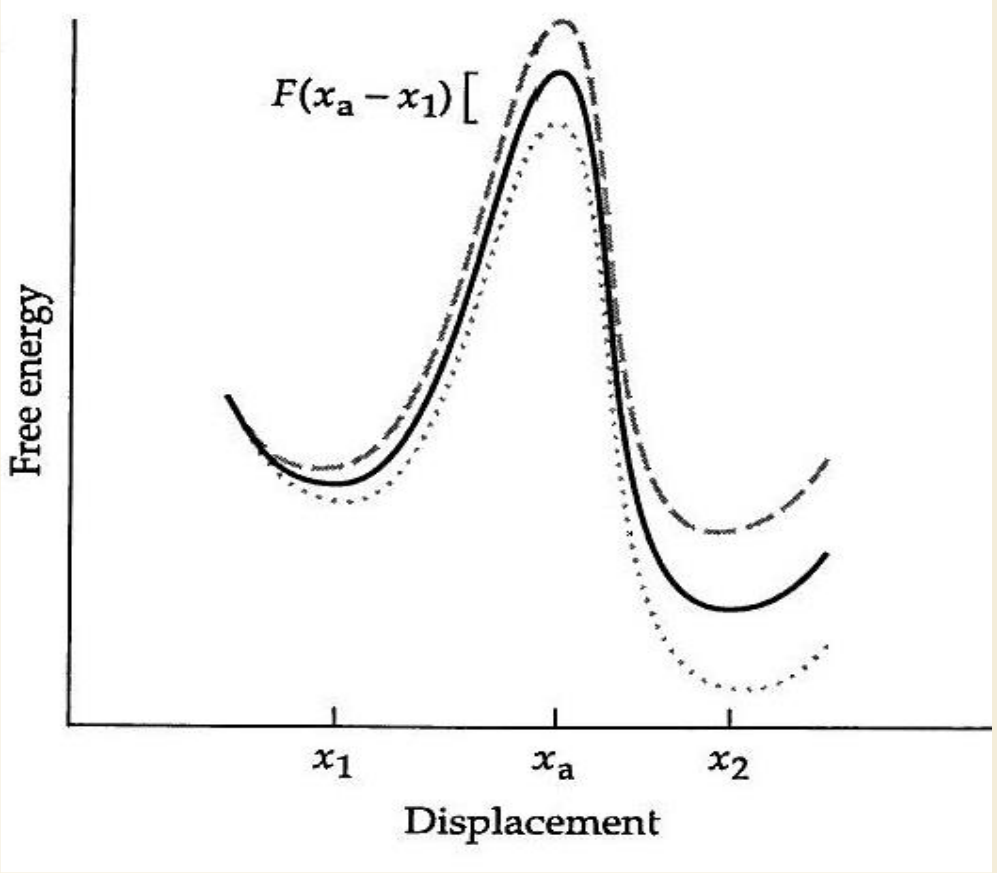
If the protein structures are very rigid and the transitions $E_1 \rightarrow E_a \rightarrow E_2$ are associated with displacements x1, xa, and x2 in the direction of the force, F, then the energies of the states will be decreased by Fx1, Fxa, and Fx2, respectively.

This implies that:

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

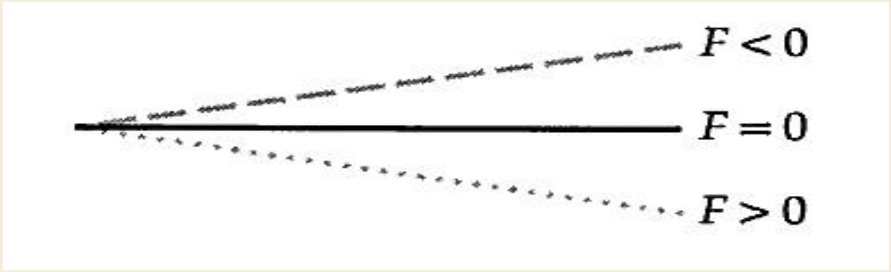
where $\Delta G_{a1} = G_{a1} - G1$ and $\Delta x_{a1} = X_{a1} - x_1$.

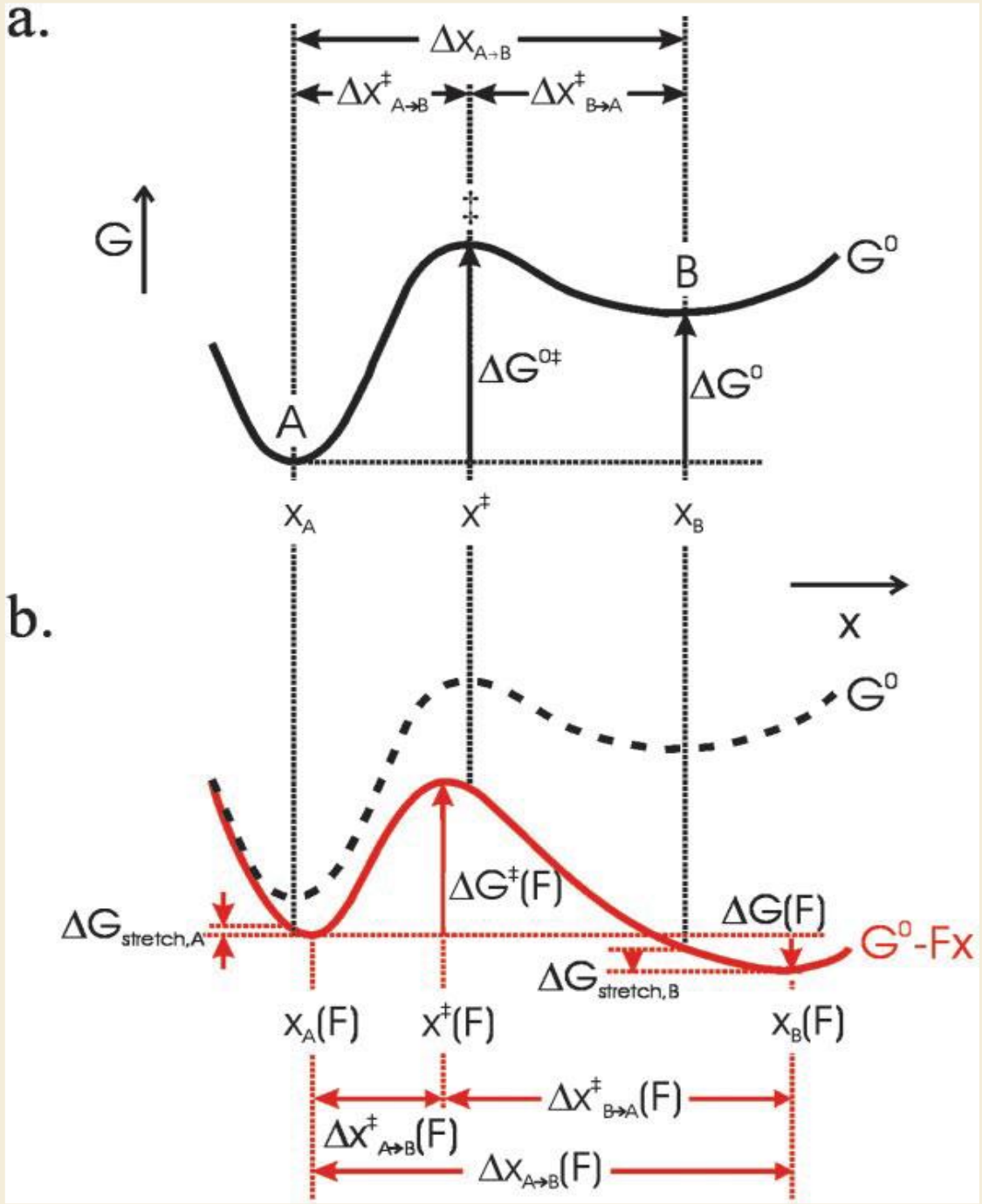
An analogous expression holds for k_{-1}.



The effect of force on the reaction rates:

tilting the free energy diagram.

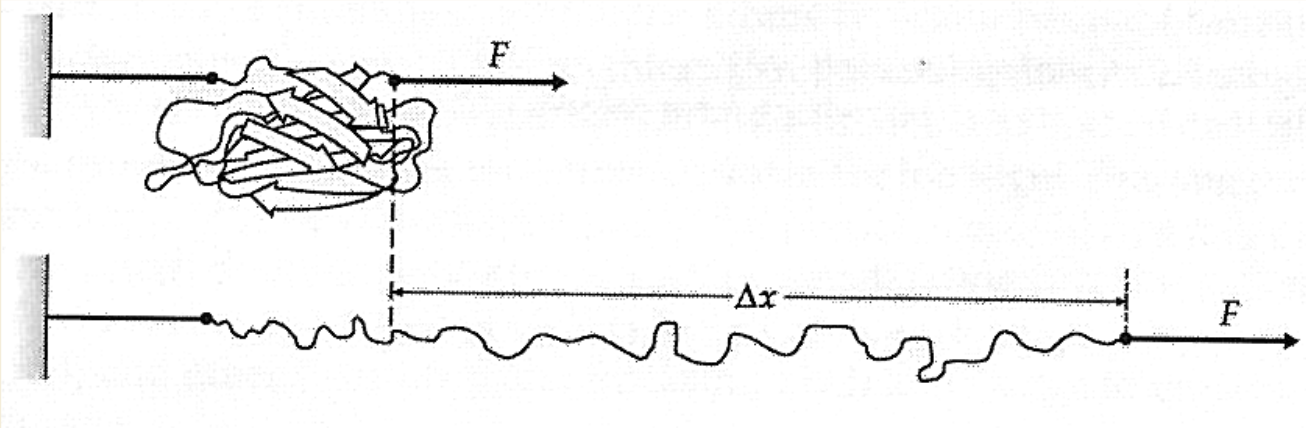


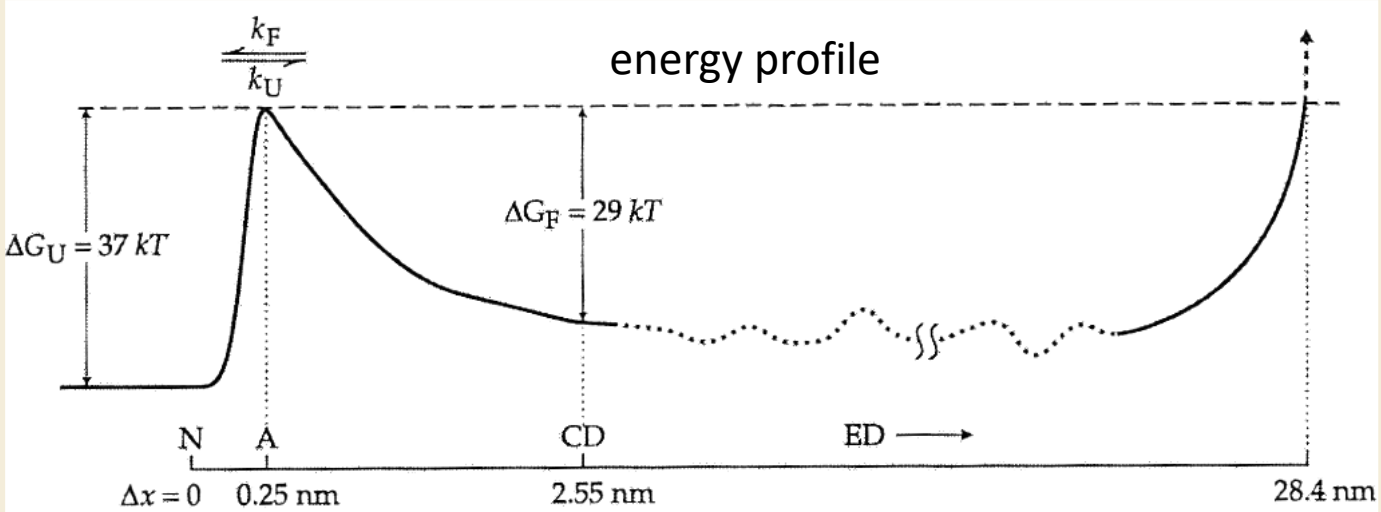


The effect of force on the free energy of a two-state system, where x represents the mechanical reaction coordinate.

(a) No applied force. (b) Red curve: positive applied force.

The application of force lowers the energy of both the transition state ‡ 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 (*xA* and *xB*) 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 Δ*Gstretch*;





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

AFM can be used to reversibly unfod immunoglobulin modules, ~ 100 amino acid domains found in a variety of proteins including the muscle protein titin.

The unfolding rate depends only weakly on force (e-fold increase per 16 pN), indicating that the transition state for unfolding is of similar length to the folded state ($\Delta x = KT/F = 0.25$ nm).

On the other hand, the folding is strongly dependent on force (e-fold slowing per 1.6 pN).

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.

But the folding of the protein requires the formation of a nearly fully folded transition state. The free energies in figure are calculated from the rates constant using Eyring rate theory.

(Carrion-Vasquez et al, 1999)

Problems 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?