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

Reference: Book_Howard_Ch_5 + others see slides

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 – bonds unbinding, --→ protein unfolding

Strength of Chemical bonds

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

1 KT ~ 4.1×10^{-21} J

NA ~ 6×10^{23}

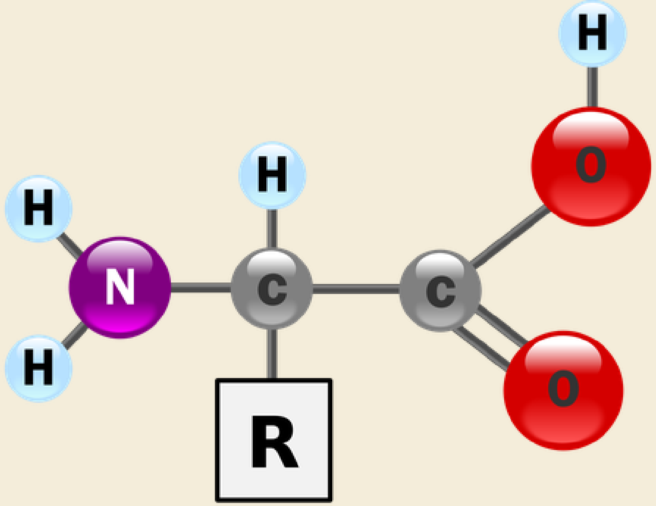
RT= KT x NA

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

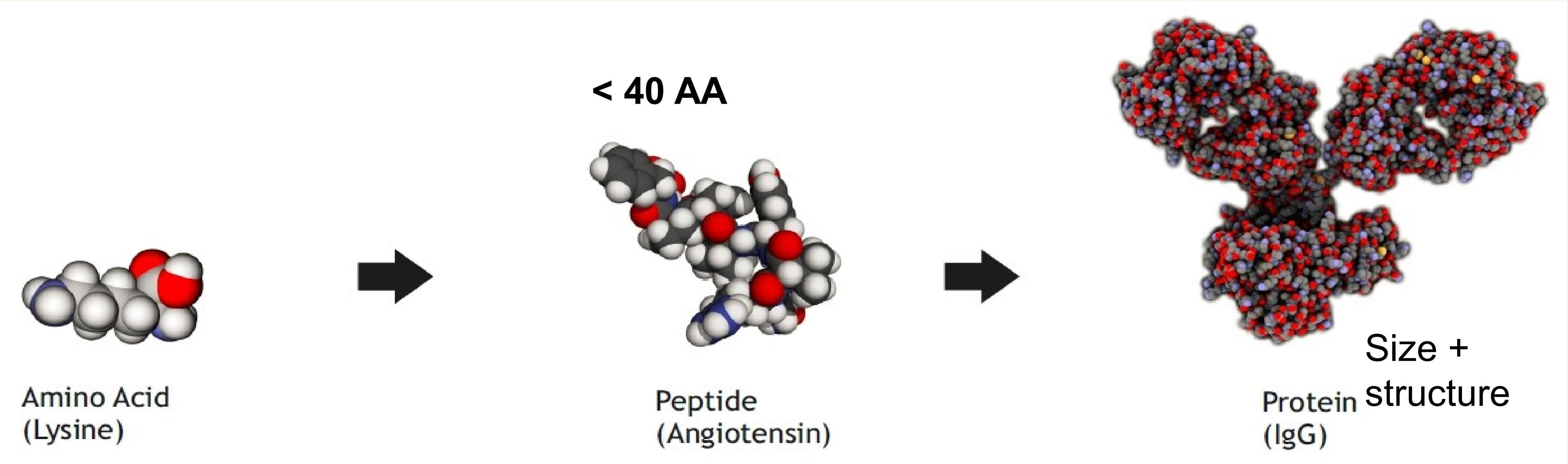
11aa of these 20 aa are synthetized by the human body

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 conponent that differs between amino acids.

Amino Acid AA

Peptide

Protein

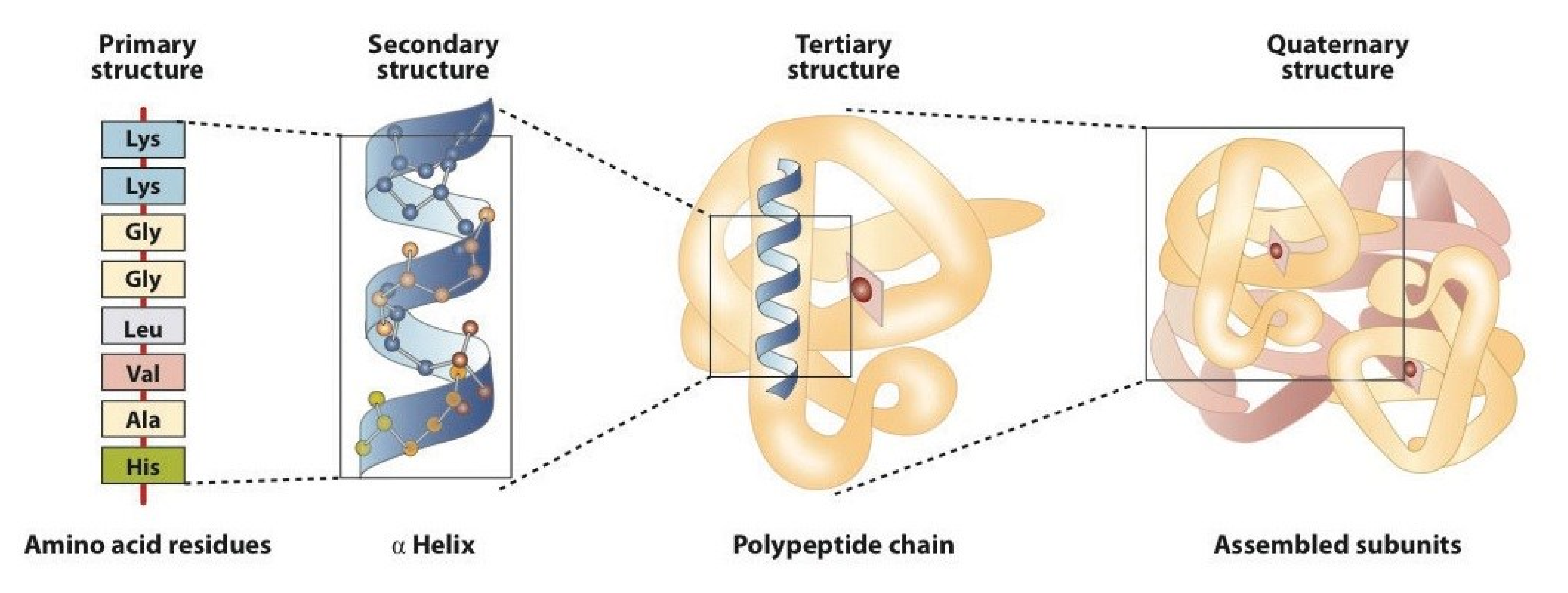


More than 20000 proteins in the human body

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

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

PROTEIN STRUCTURE



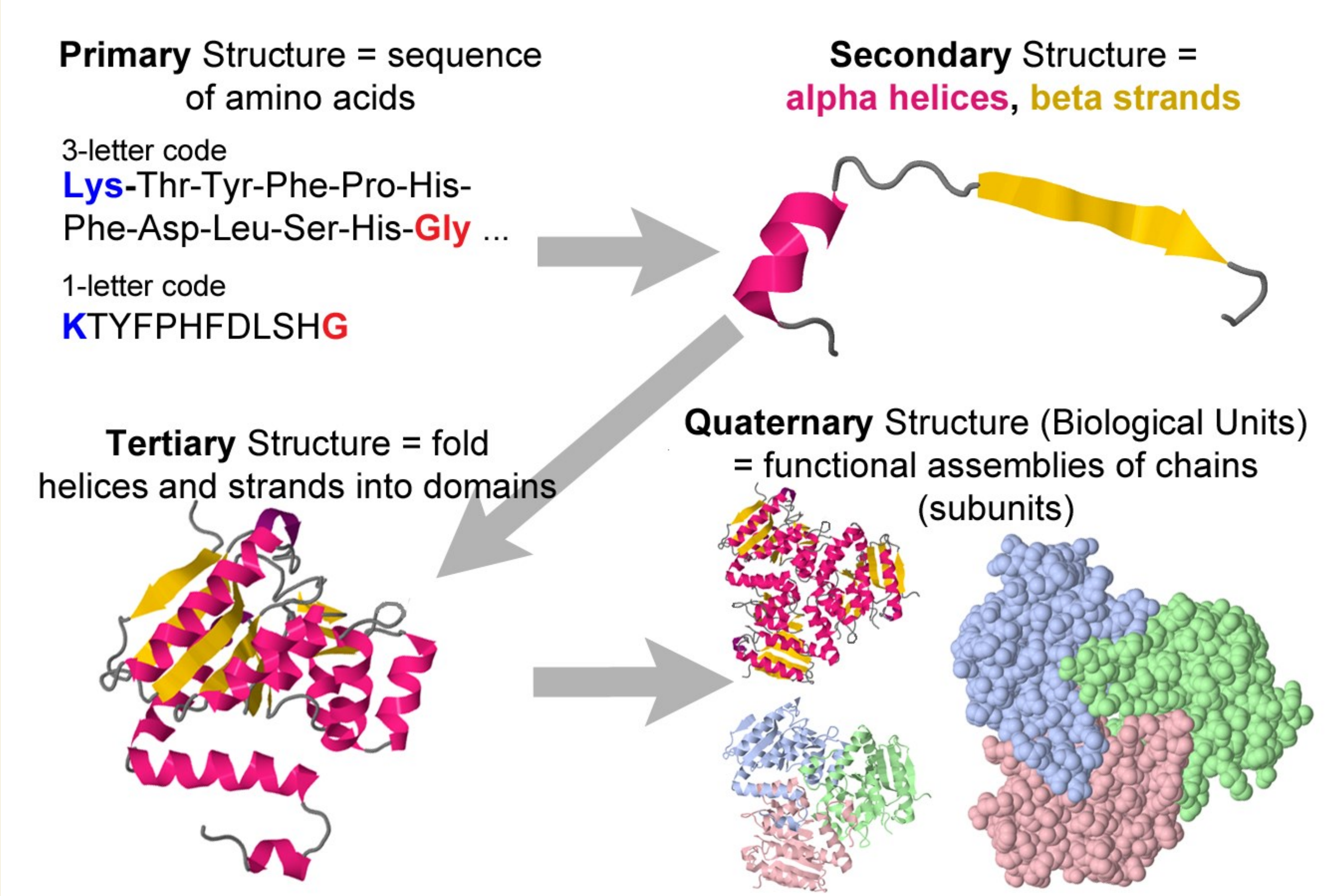
The *primary structure* consists of the specific amino acid sequence.

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.

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

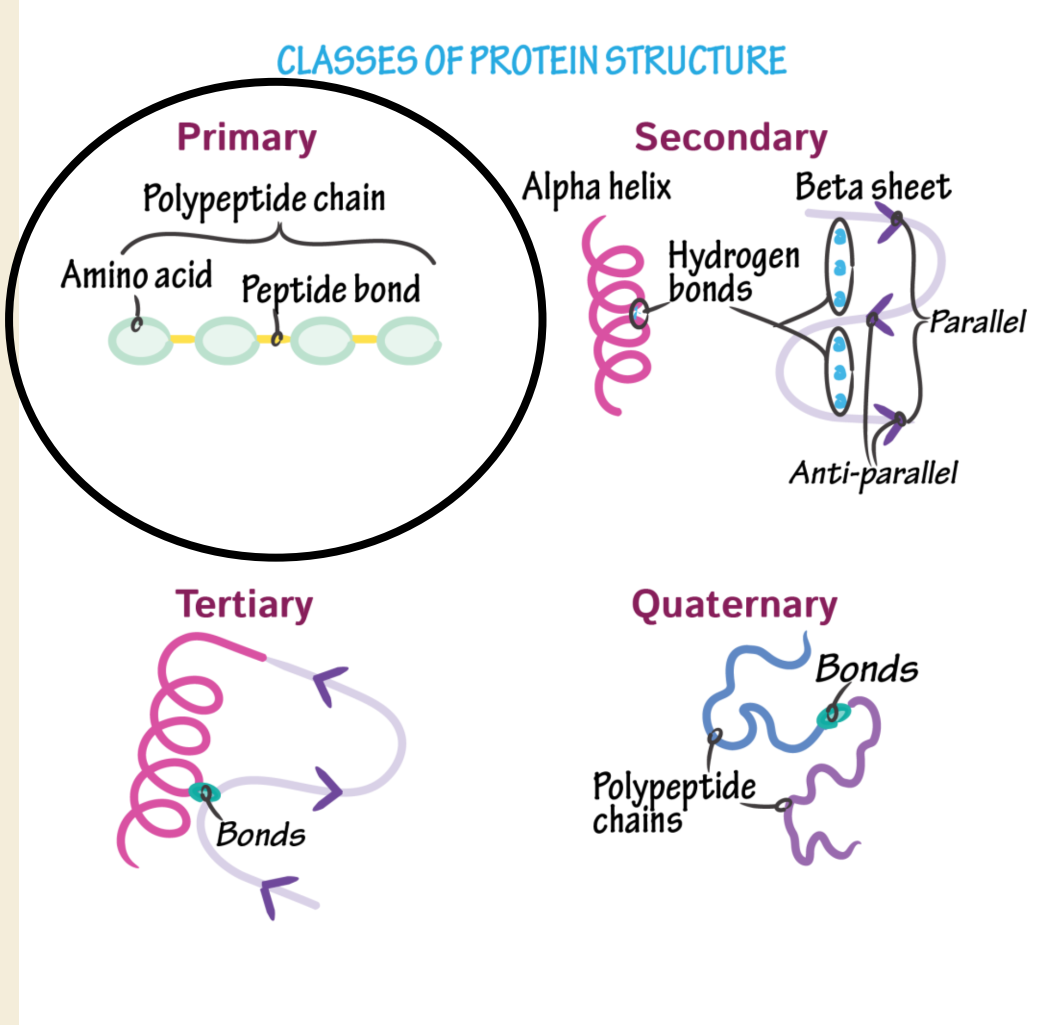
Quaternary Structure (Biological Units)
= functional assemblies of chains
(subunits)

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.



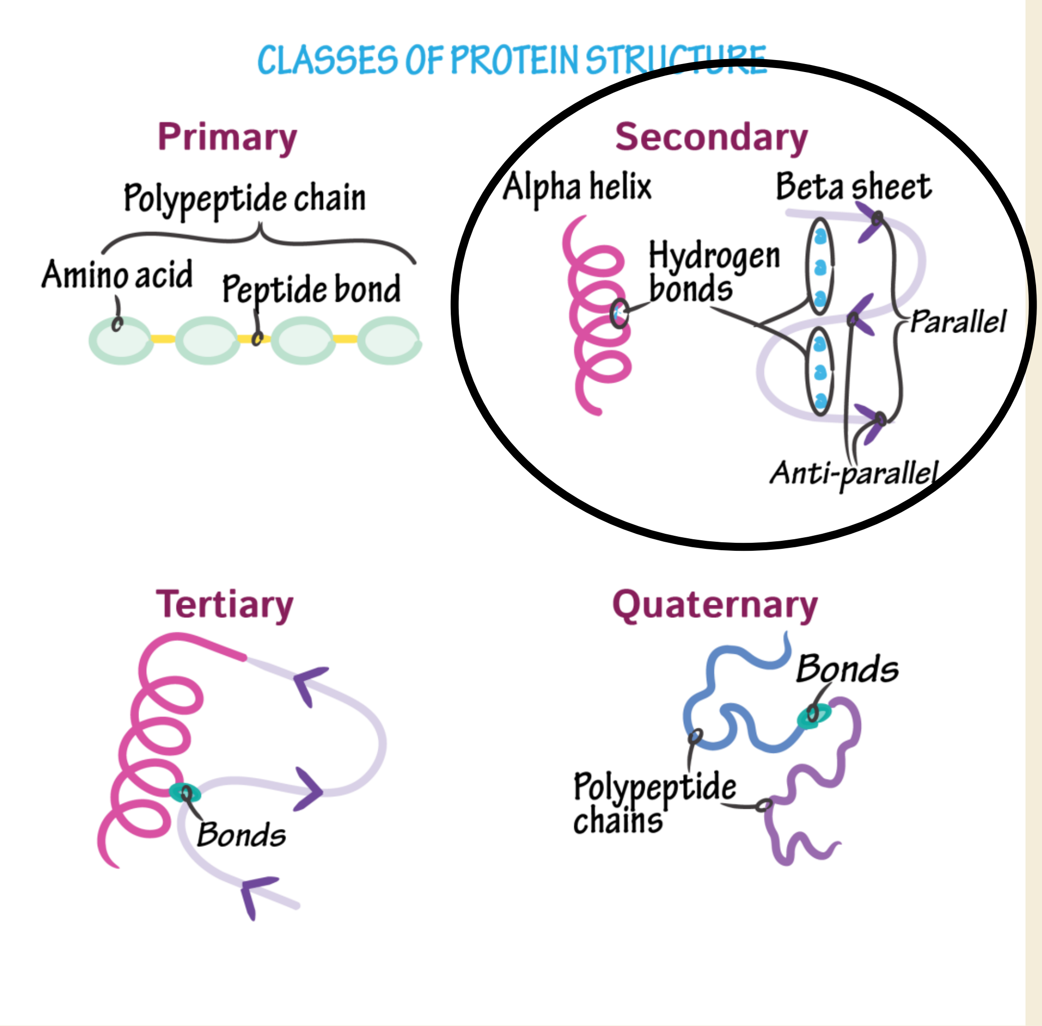
SECONDARY PROTEIN STRUCTURE

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.

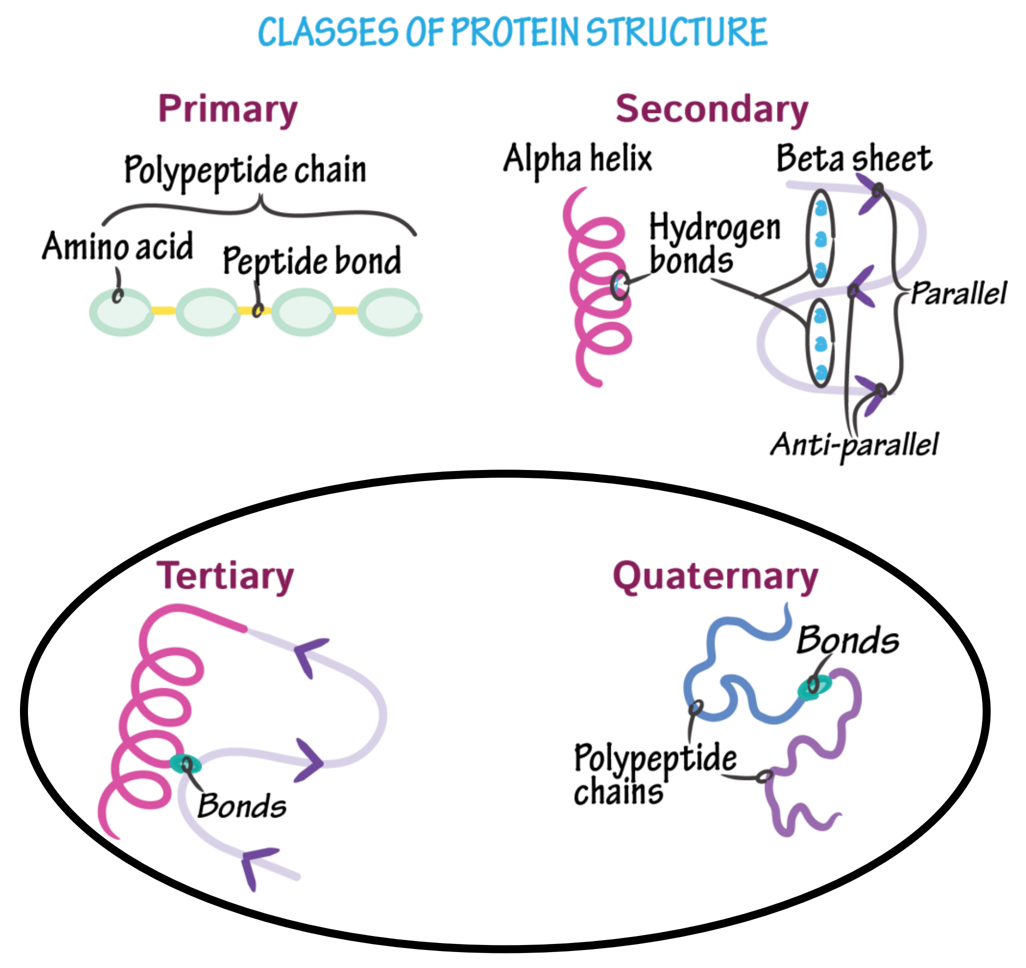
The following additional forces stabilize these structures:

-Hydrophilic interactions.

-Electrostatic interactions.

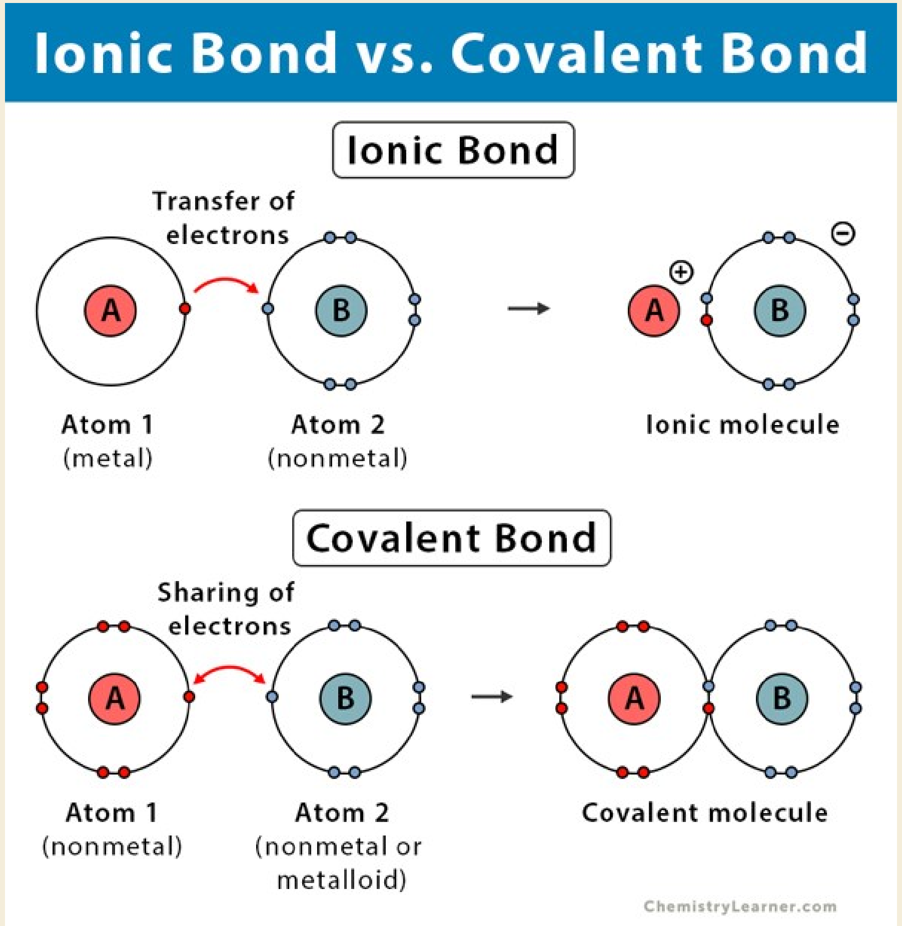
-Hydrogen bonds between side chains.

-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.

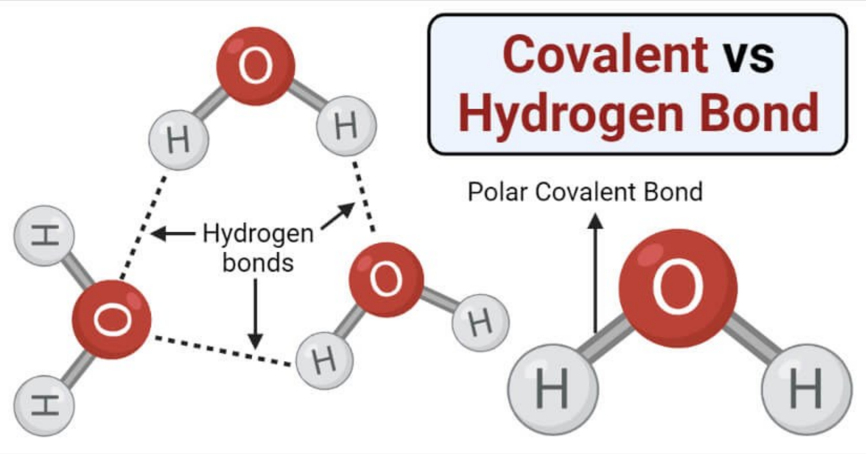
https://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/bonding/chymo.html#top

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).



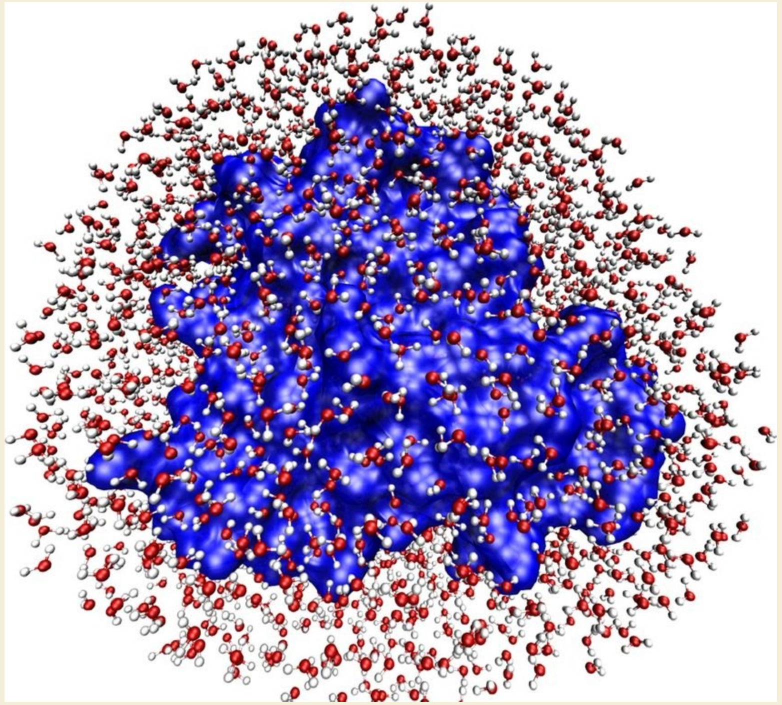
C. Water Shells and Polar Surface Residues

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.

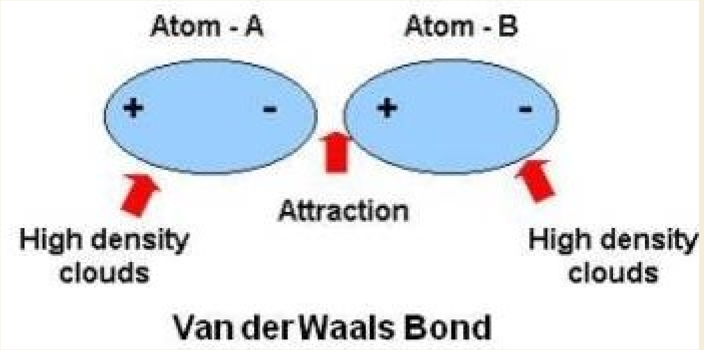
There are six amino acids with side chains that are polar: serine (Ser), threonine (Thr), cysteine (Cys), asparagine (Asn), glutamine (Gln), and tyrosine (Tyr).

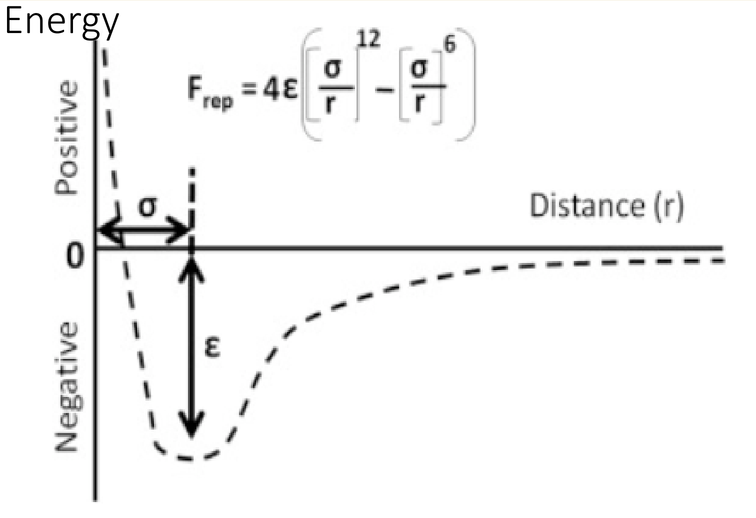


The hydration shell of myoglobin

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.

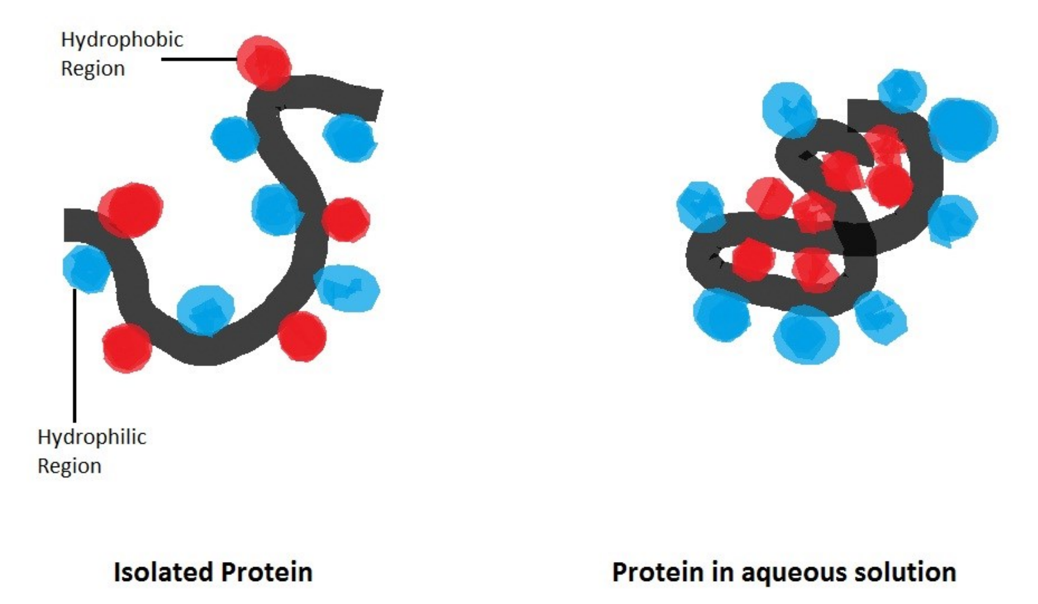
Hydrophobic Interactions

Strength ~ 5 KT / 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

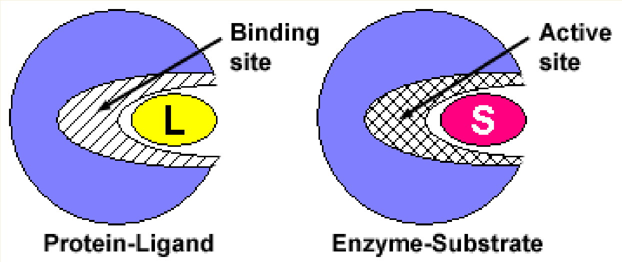
The ligand binds to the protein by weak interactions, inducing a conformational change of the protein. The physical action of the ligand changes the overall shape of the entire structure without breaking or forming new chemical bonds.

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.

https://biologydictionary.net/ligand/

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.

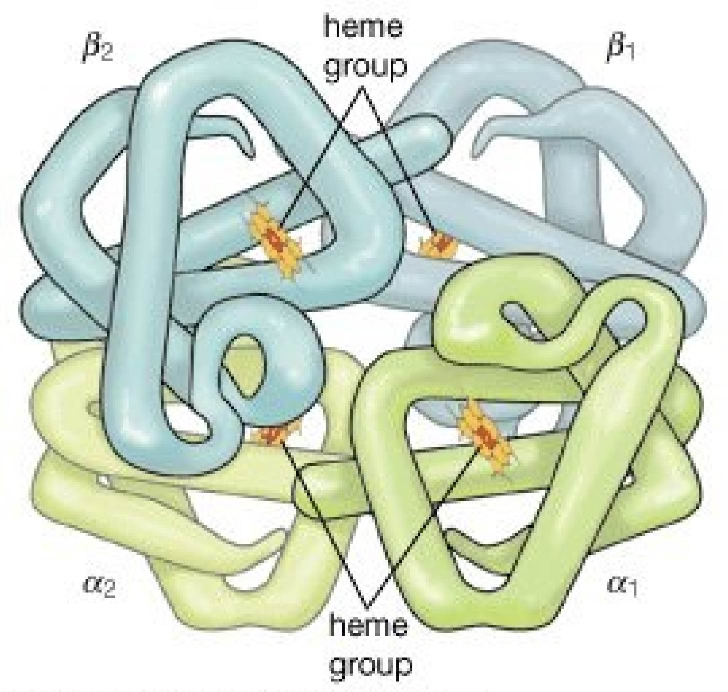
Hemoglobin is the major blood protein responsible for transporting oxygen in humans. Hemoglobin picks up oxygen in the lungs and releases it in capillaries. Release is induced by a conformation change of hemoglobin, which is produced by a lower pH level because of CO2 increase and because of red blood 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 x 10^9 RBC / mL blood; 0.21 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 the attachment and detachment of the oxygen molecules.

Note: another ligand is the carbon monoxide. CO has a higher affinity for hemoglobin than O2 has. Once CO is bound to the hemoglobin, it won't come off. This means that someone exposed to large amounts of CO will soon have all their hemoglobin saturated by the wrong ligand. Their body will have no ability to transfer oxygen to the brain and body tissues.



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Examples of a Ligand

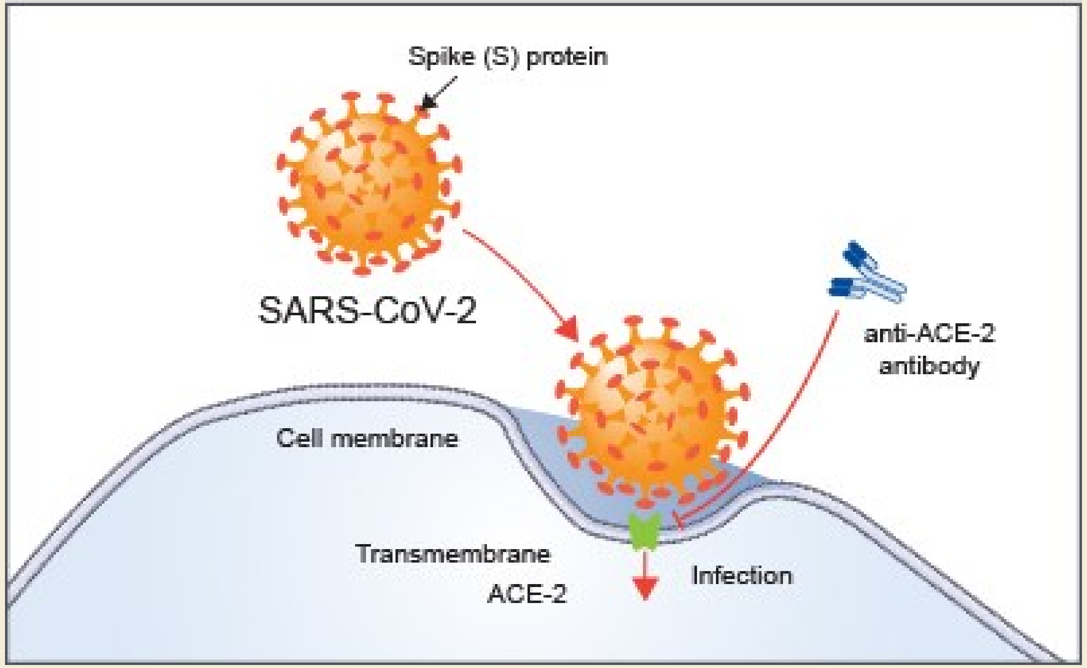
Dopamine

Dopamine is a ligand used heavily in the brain. It is a neurotransmitter. When the brain (dopaminergic neurons) releases it and the dopamine receptors are reached, signaling cascades are triggered inducing the sensation of a pleasure coming from success. In other words, dopamine is tied to the sensation of motivation. 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”, sensation / situation 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.

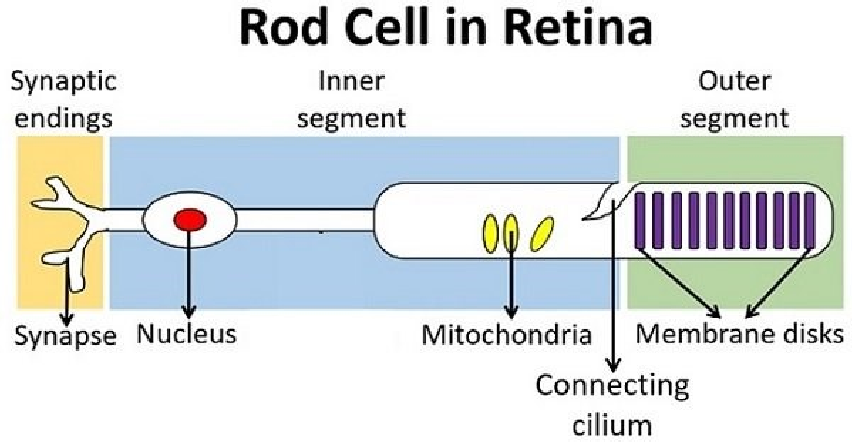


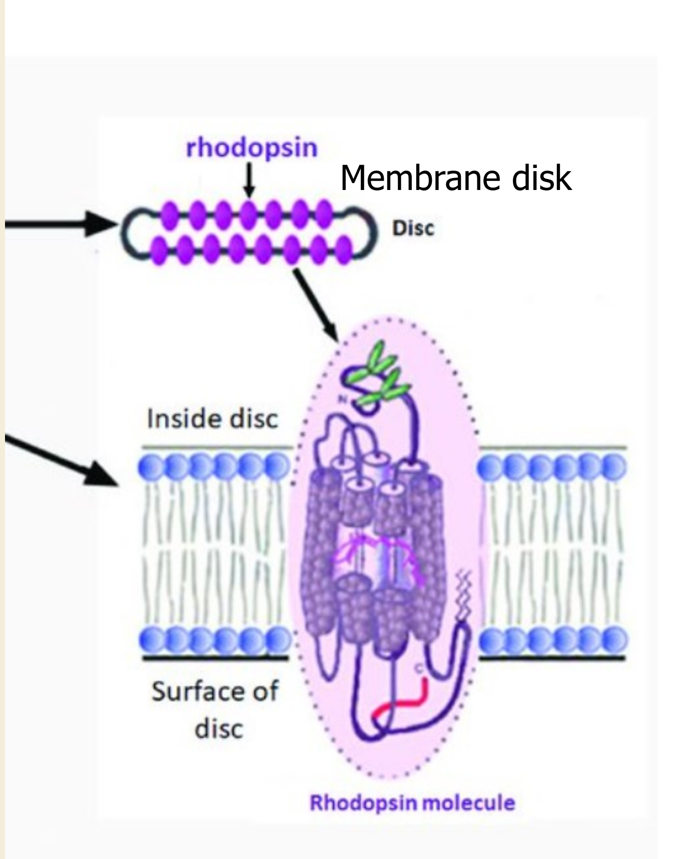
https://www.rndsystems.com/resources/articles/ace-2-sars-receptor-identified

Chemical forces

Examples of a Ligand

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





Rod cells are light sensitive photoreceptor cells in the retina of the eye that work better at low light intensity levels (dark/night), while cone cells work at higher light levels.

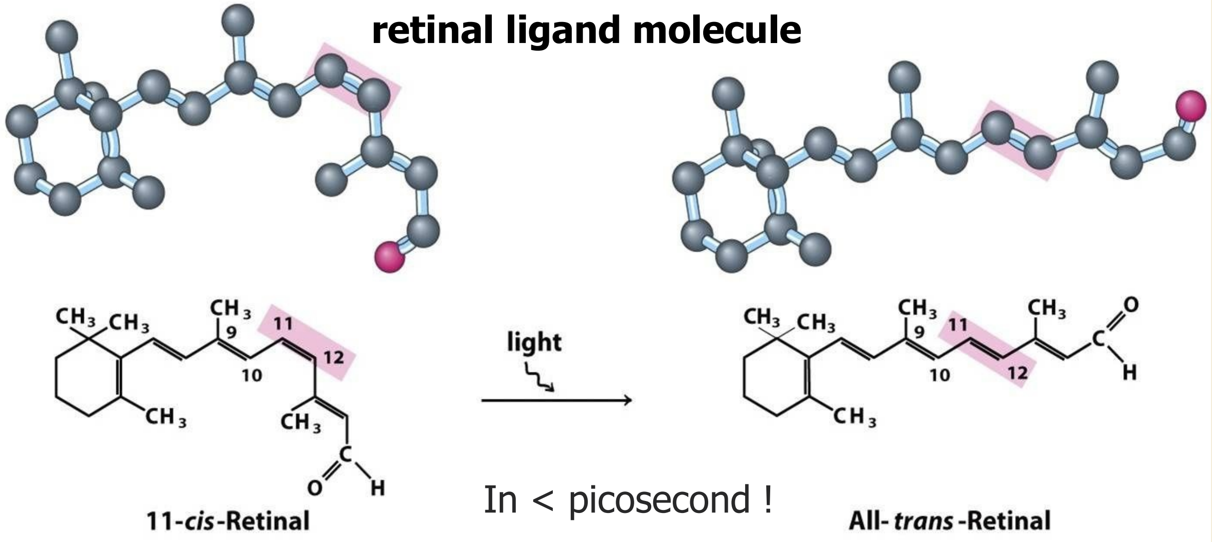
Rhodopsin is a light-sensitive receptor protein involved in visual phototransduction.
It is is a biological pigment found in the rods of the retina and is a G-protein-coupled receptor.
Rod (and **cone**) are photoreceptor cells in the retina.

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

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.

retinal ligand molecule



In < picosecond !

Figure 1-18b
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

Cis to trans isomerization

Isomerization process in vision:

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

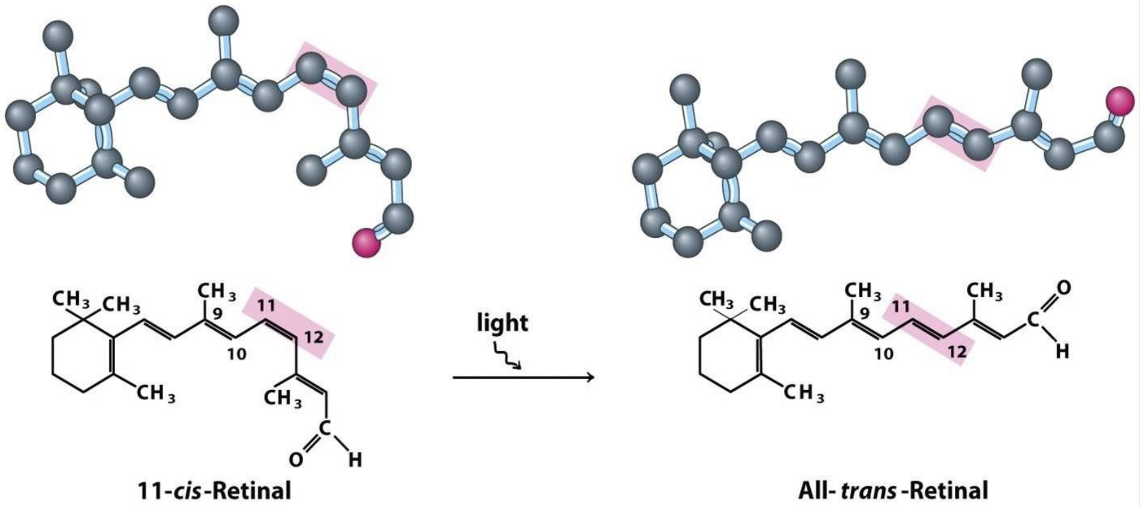


Figure 1-18b
Lehninger Principles of Biochemistry, Fifth Edition
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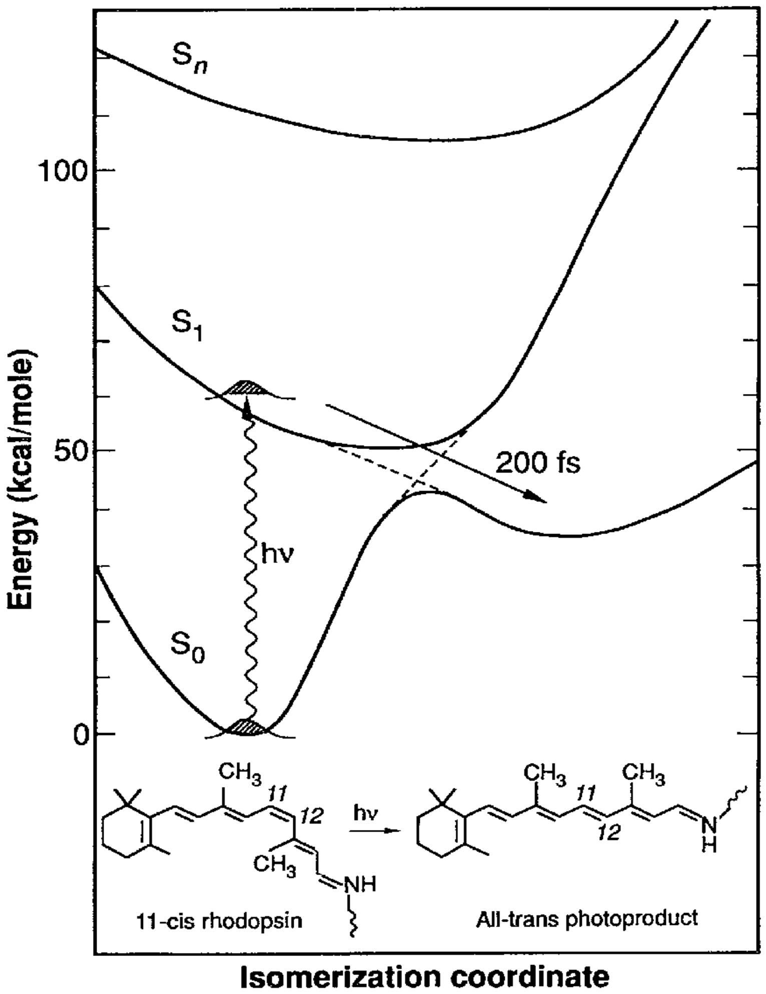
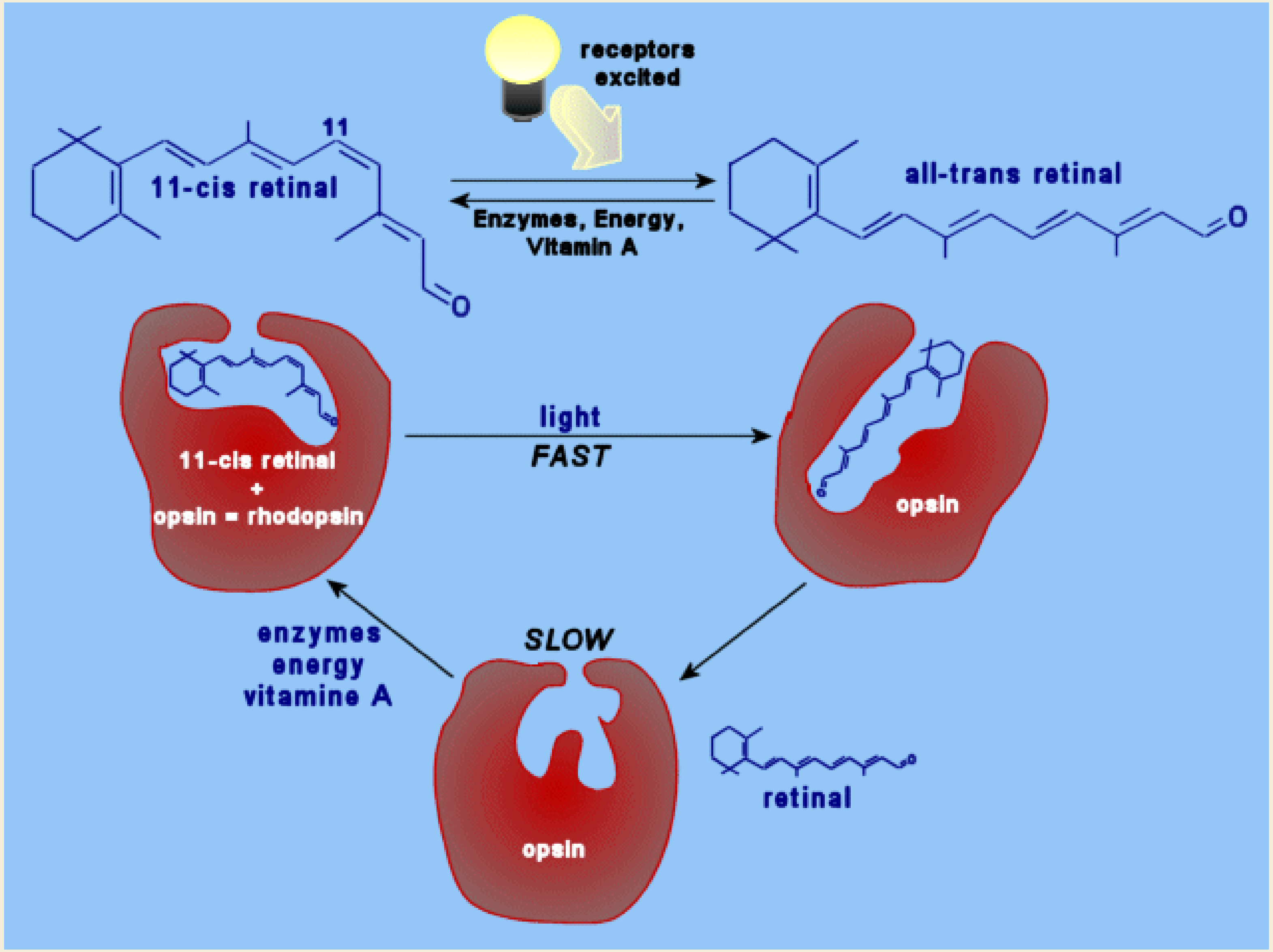


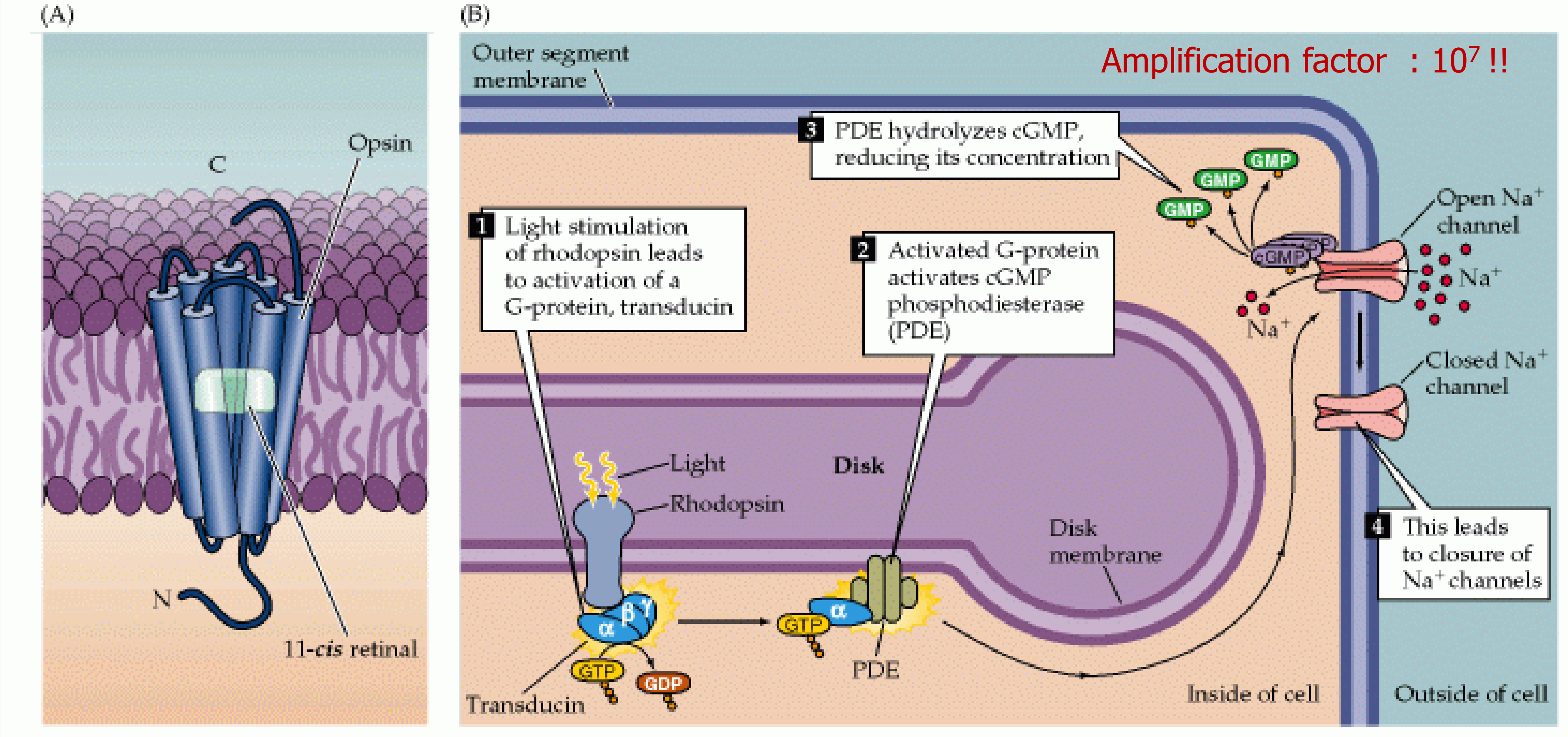
Fig. 1. Schematic ground-state and excited-state potential energy surfaces for the 11-*cis* → 11-*trans* isomerization in rhodopsin, adapted from (*14*). The reaction path of the photoisomerization is indicated by the nonadiabatic potential surfaces (broken lines).

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

Reversible cycle of retinal isomerization in rhodopsin



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.

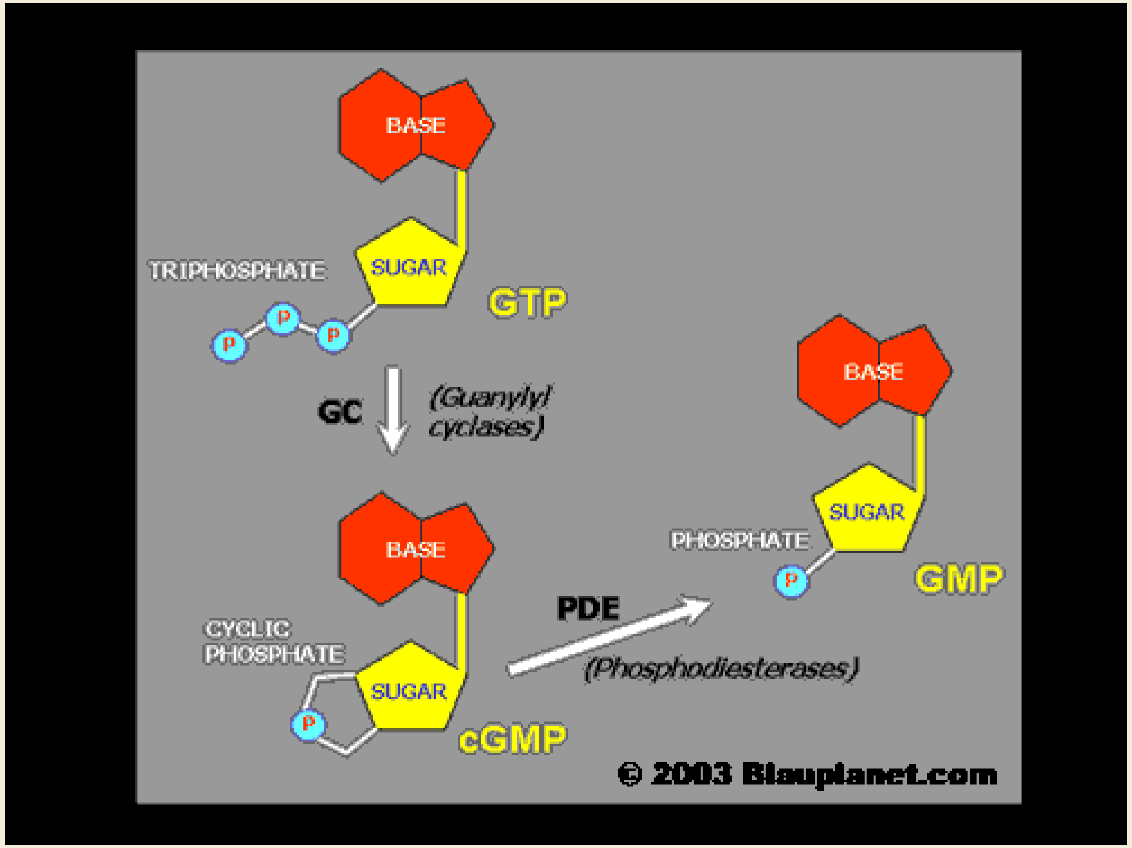


Cyclic guanosine monophosphate (cGMP)

Phosphodiesterase (PDE)

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

Phosphodiesterase (PDE) and cyclic Guanosine MonoPhosphate (cGMP)



Cyclic guanosine monophosphate (cGMP) is a cyclic nucleotide derived from guanosine triphosphate (GTP).

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

Lab Experiment Stimulating a single Rod cell

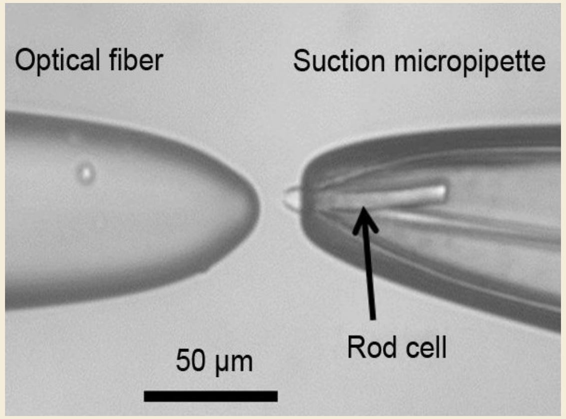
Stimulating Rod cell

with light and measure current

A single rod cell is illuminated and the current

generated is measured in the micropipette

Lab exp.



Chemical forces

How 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 $E1 \rightleftharpoons E2$?

Chemical equilibria and the effect of force on chemical equilibria

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:

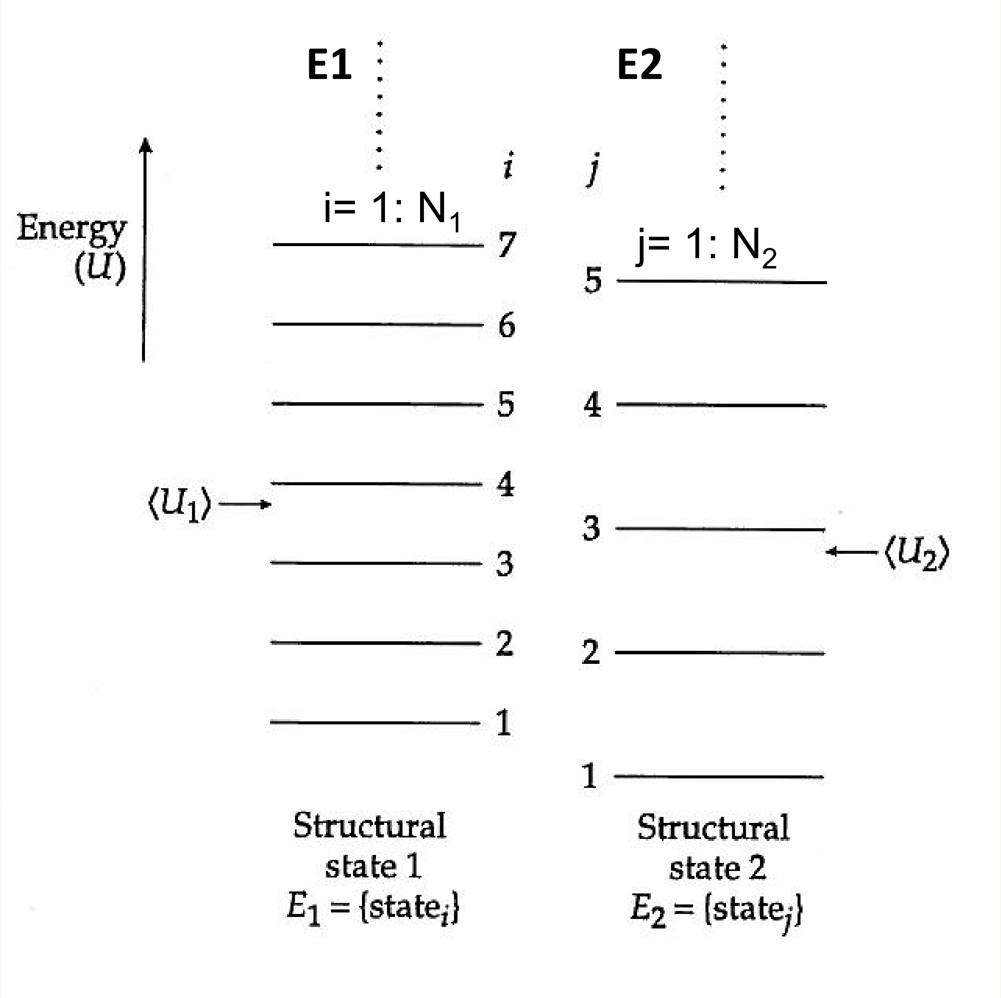
folded vs unfolded protein, closed vs open ion channel, ligand – receptor unbound vs bound

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.

The probability of finding the protein in one of the two (structural) states

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:

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

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

$$p_1 = p(E1) = \sum^{N_1} p_i = Z_1 / Z \qquad p_2 = p(E2) = \sum^{N_2} p_j = Z_2 / Z$$

$$P_2 / P_1 = Z_2 / Z_1$$

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

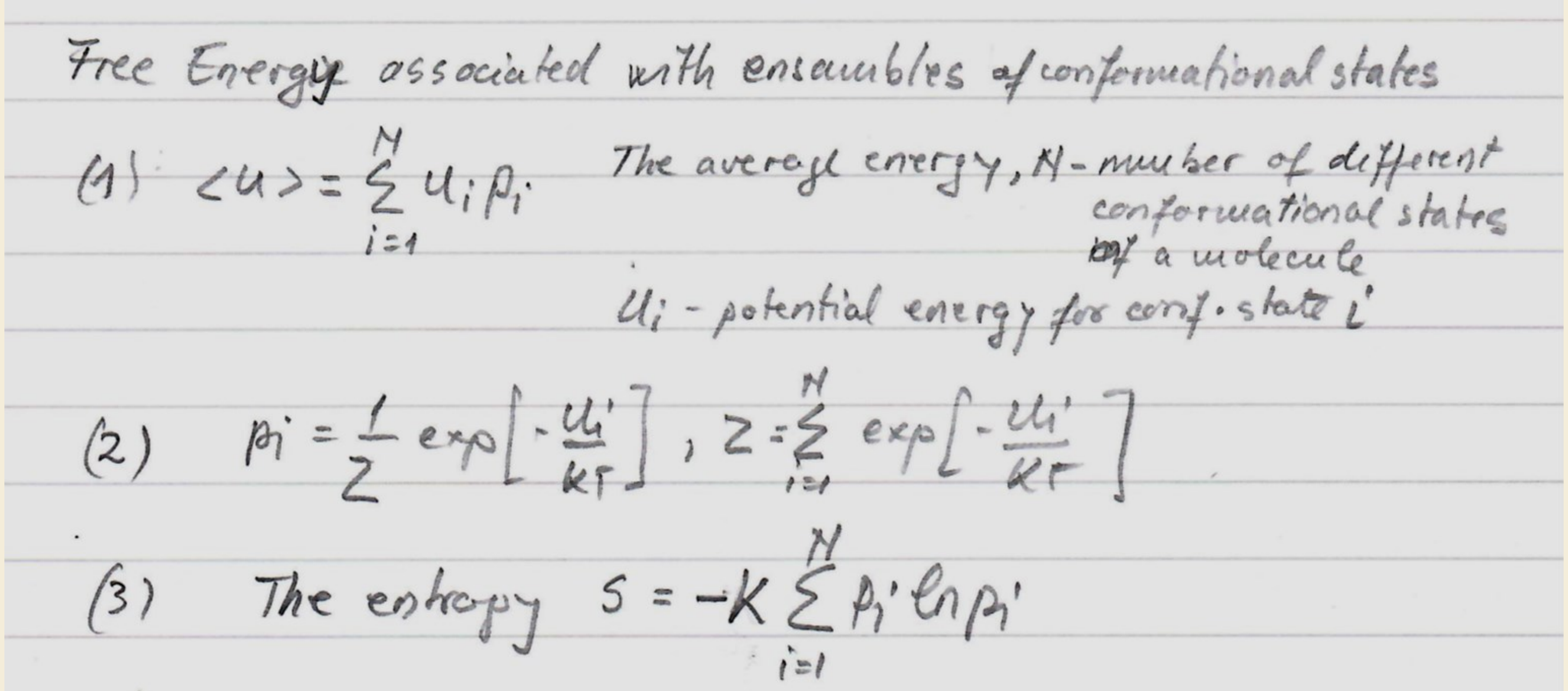
Free Energy

For a structural state ***E, which is an ensamble of states with energies Ui,*** the free energy ais defined 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 Ω*** with Ω the number of microscopic configurations (the larger the number of conformations in an ensemble, the greater the entropy ***S***)



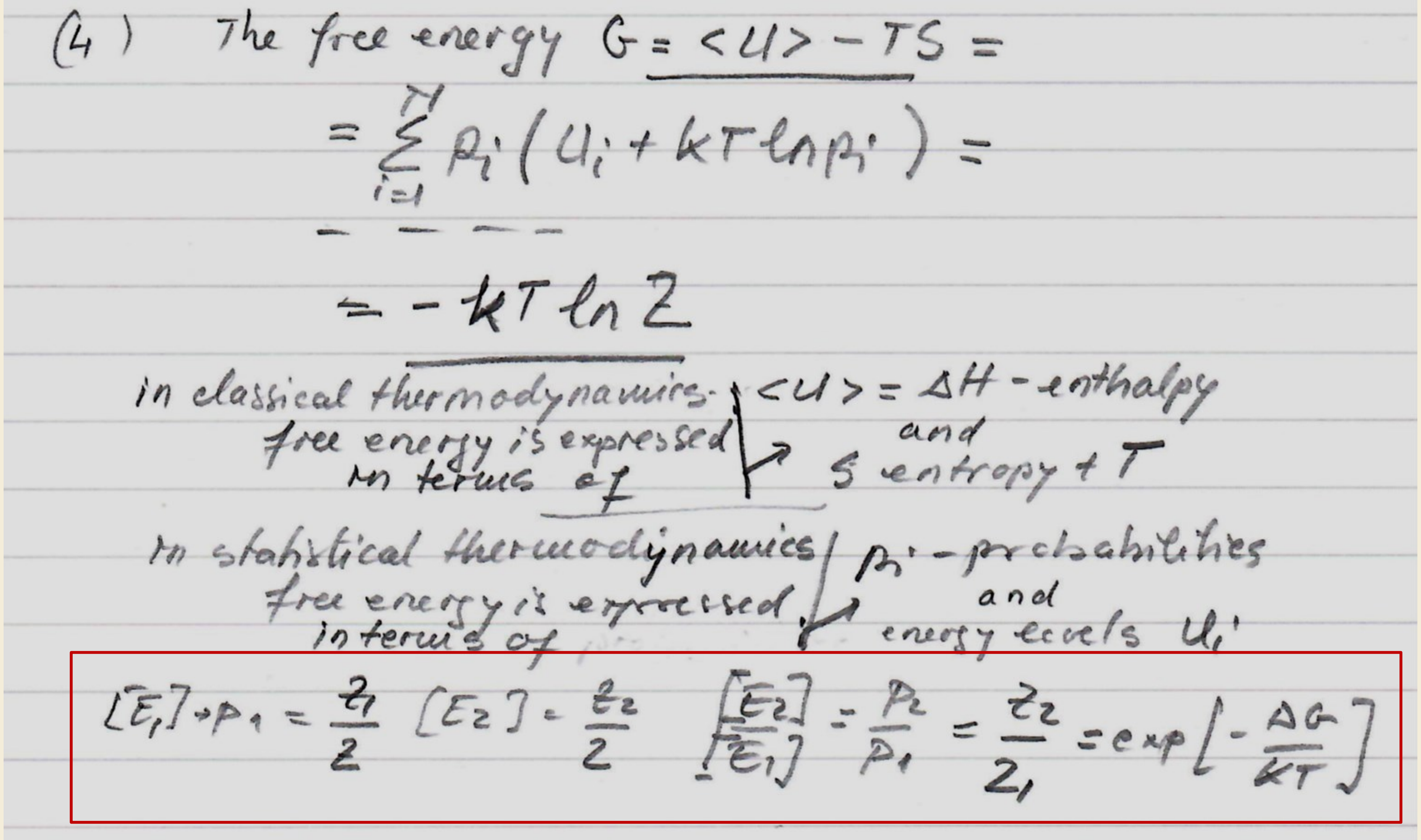
Free Energy associated with ensambles of conformational states

(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 $S = -K \sum_{i=1}^{N} p_i \ln p_i$



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

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

$$= -kT \ln Z$$

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

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

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.

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

$$\Delta G = G_2 - G_1$$

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

K_{eq} – equilibrium constant

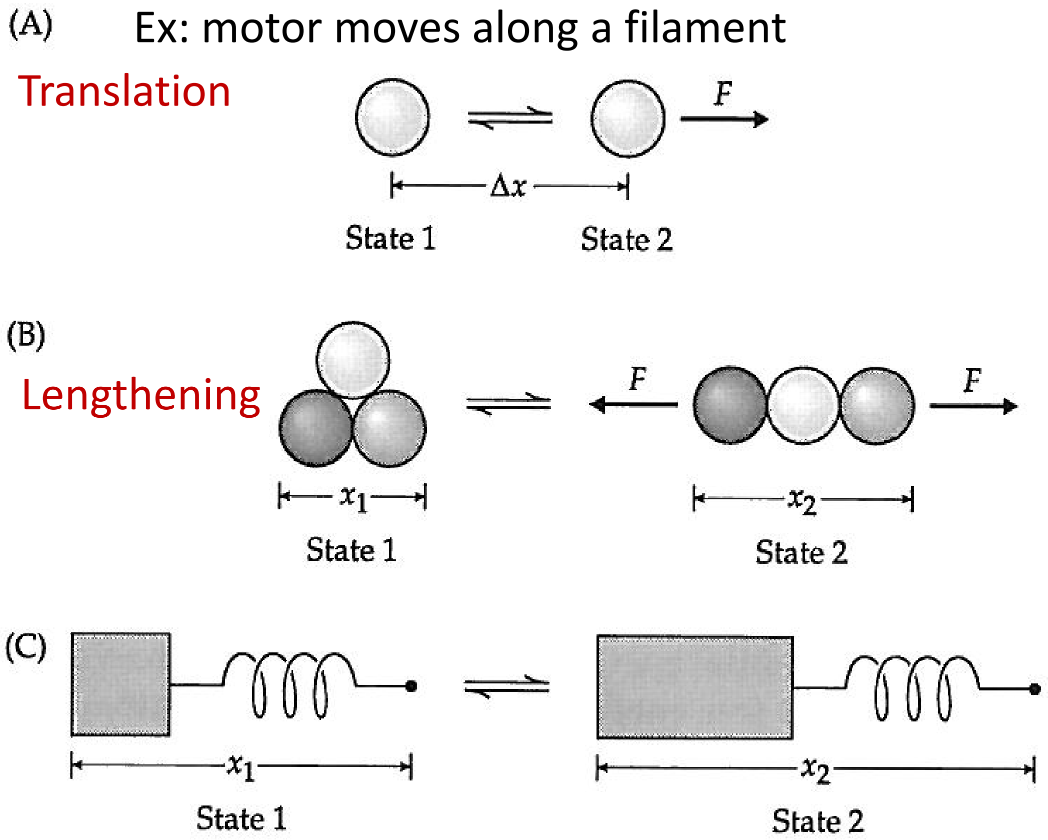
Law of Mass Action

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 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



Ex: motor moves along a filament

Translation

Lengthening

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

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

Chemical equilibria and the effect of force on chemical equilibria

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

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

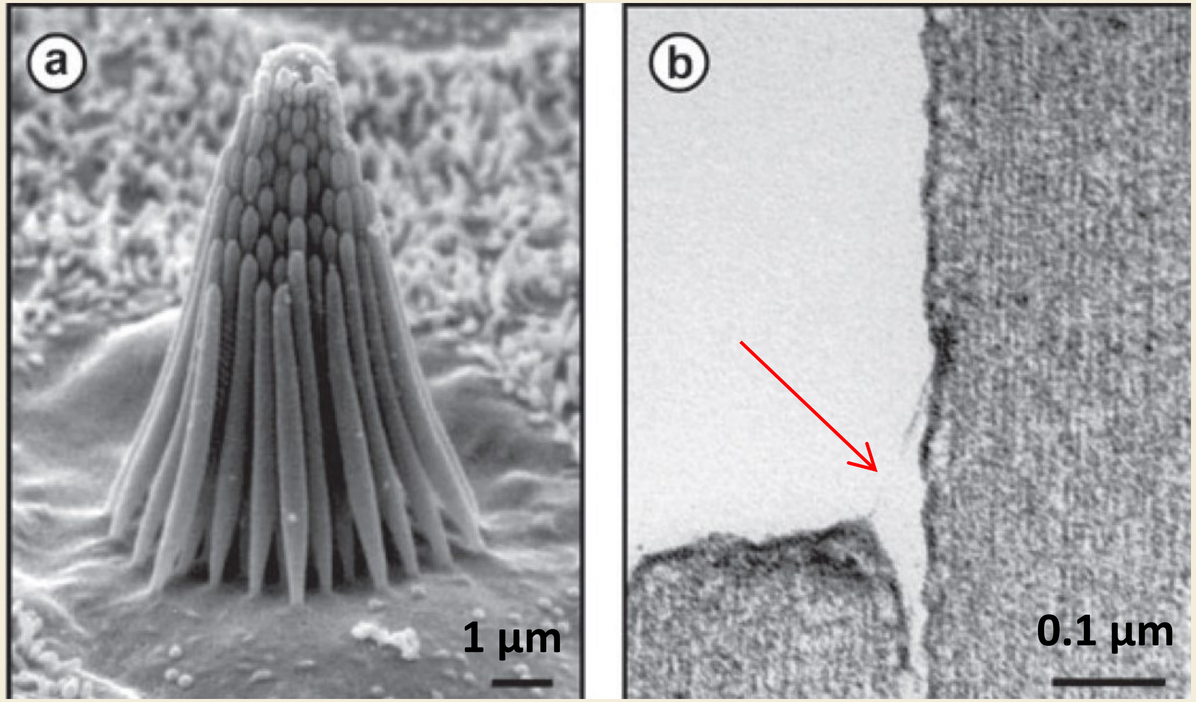
An external force couples to a structural change if it is associated with a length change in the direction of the force.

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** !

Example of how the forces modulate the state of a protein.

Mechanically sensitive ion channel in hair cells.



Hair cell anatomy:

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

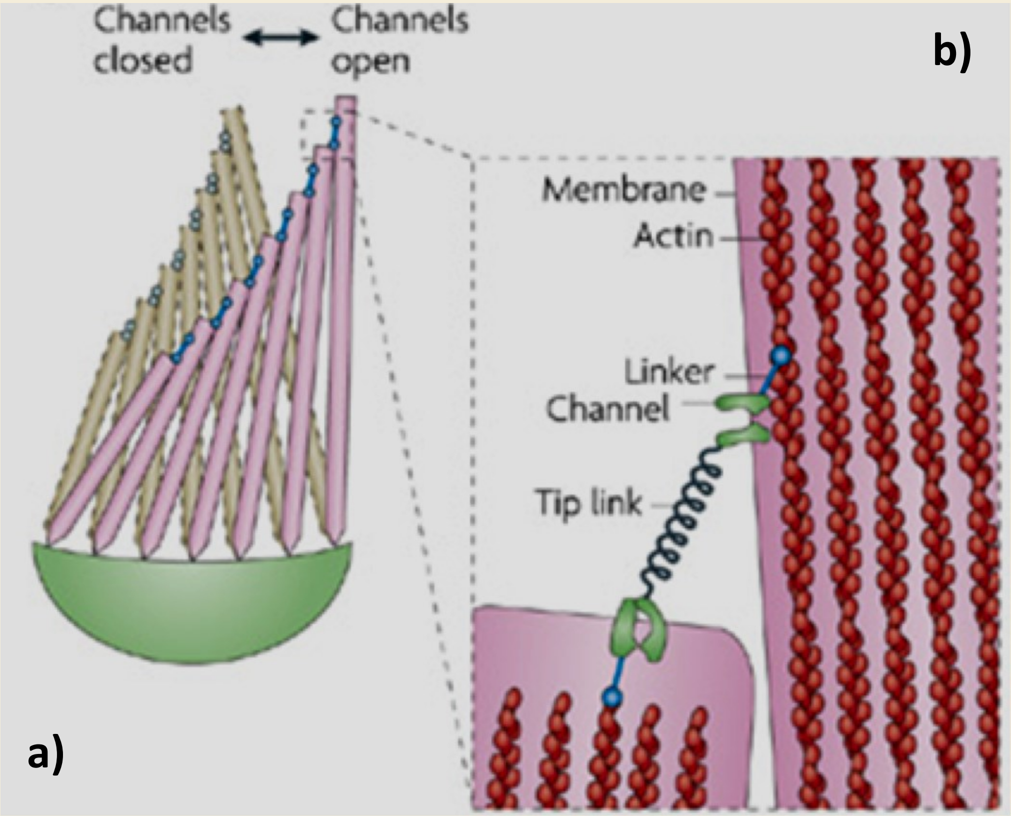
(*b*) Two stereocilia and the tip link extending between them

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

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 <u>resting</u> (yellow) and <u>deflected</u> (pink) <u>configurations</u>.

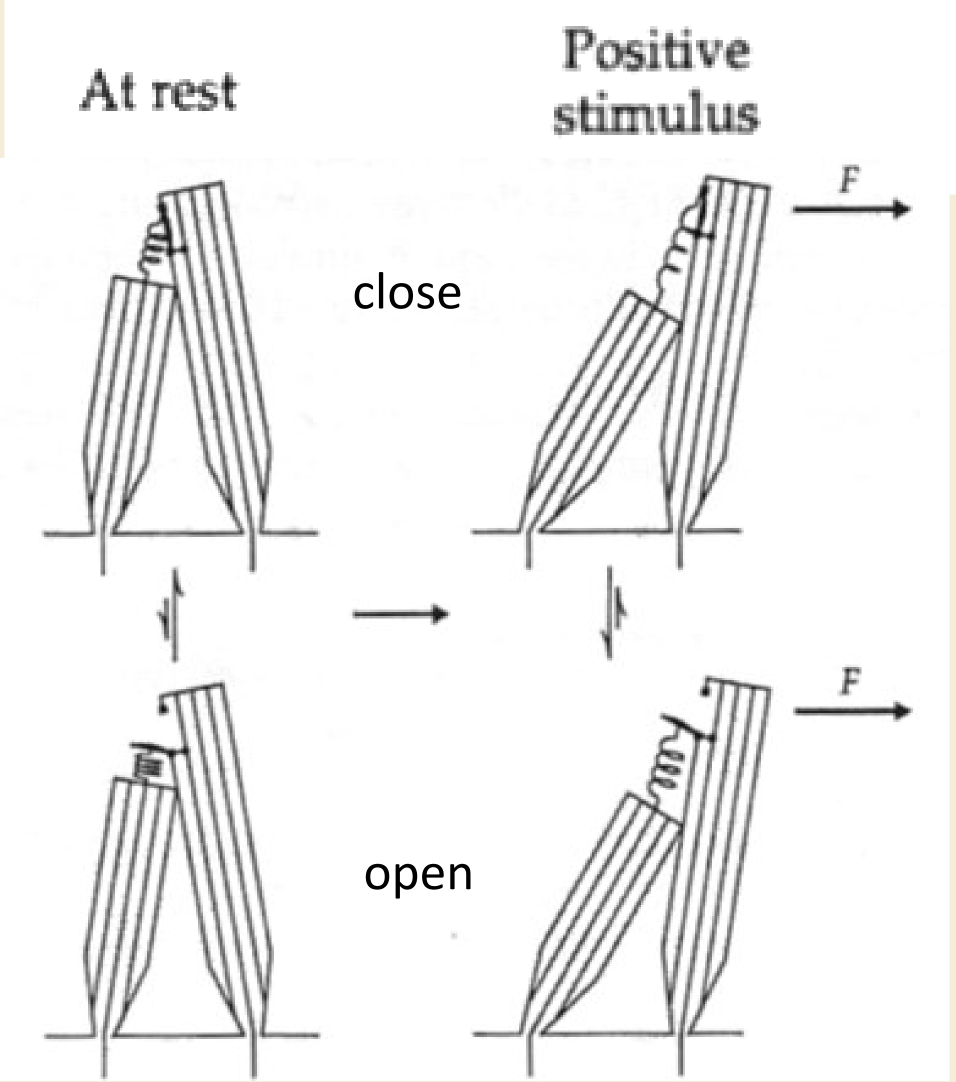
b) Deflection, i.e. shearing of the stereocilia relative to each other, causes the tip links to pull directly on K^+ (and Ca^{2+}) channels in the stereocilia, causing the channels to open.

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

Neuron 2005, www.cell.com/neuron/pdf/S0896-6273(05)00881-0.pdf

Open / close probabilities of the Mechano Sensitive Ion Channel



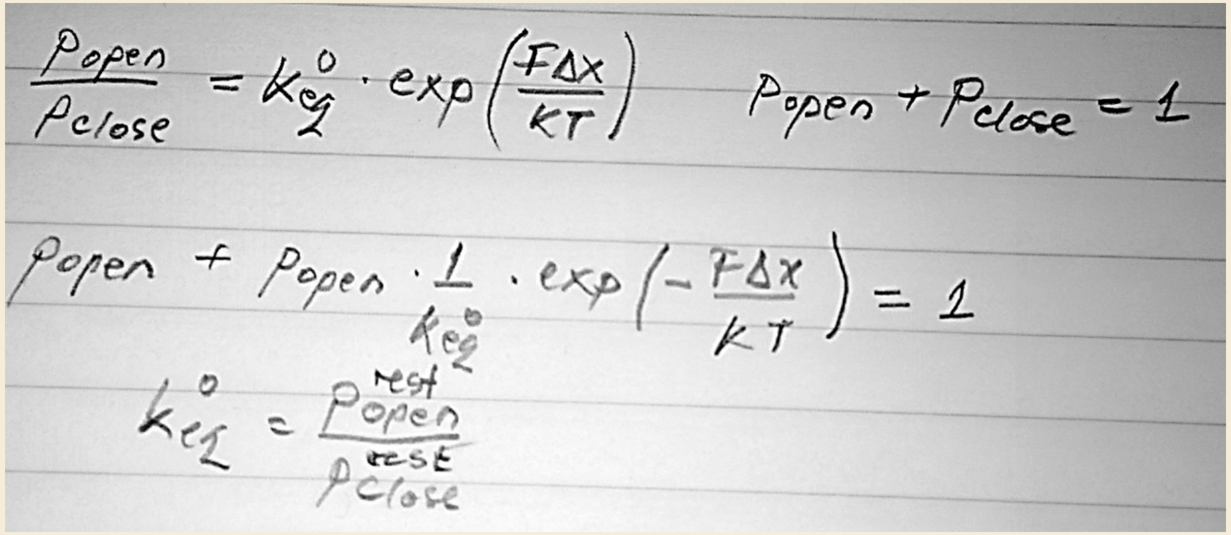
External Force → deflection of hair bundle → shear between adjacent stereocilia → tension in the elastic tip link

→ 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.



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

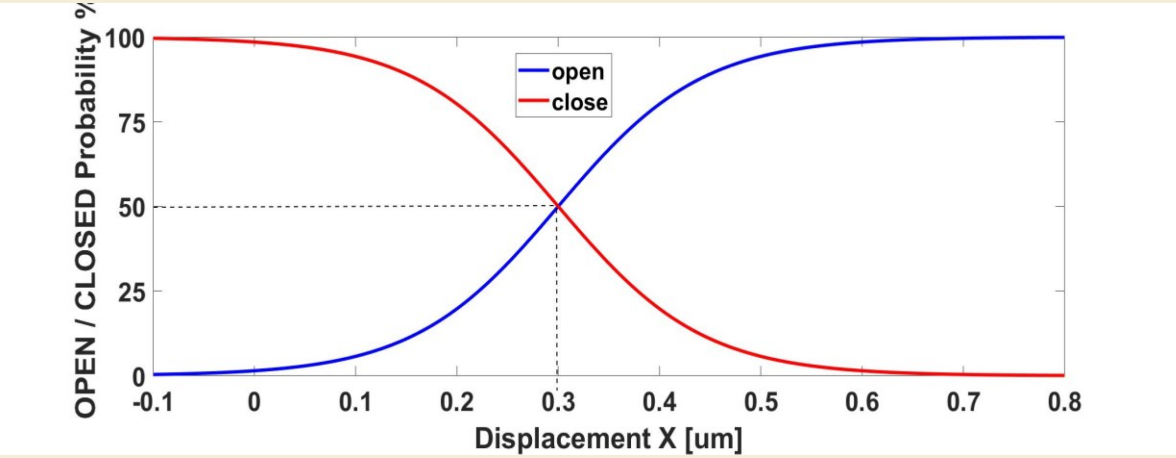
$$P_{open} + P_{open} \cdot \frac{1}{k_{eq}^{0}} \cdot \exp\left(-\frac{F\Delta x}{kT}\right) = 1$$

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

Open / close probabilities of the Mechano Sensitive Ion Channel – with Force

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= 2pN/nm

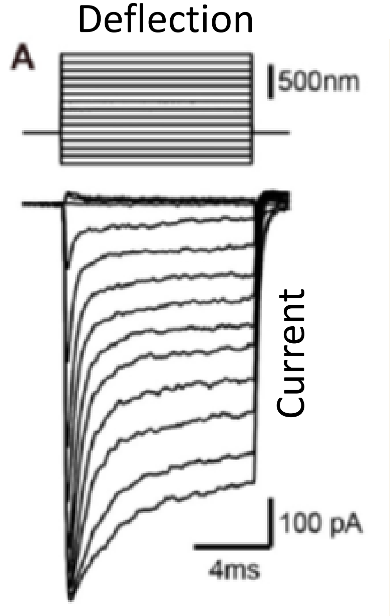
X – the displacement of the hair bundle (X=-0.1 – 1 um),

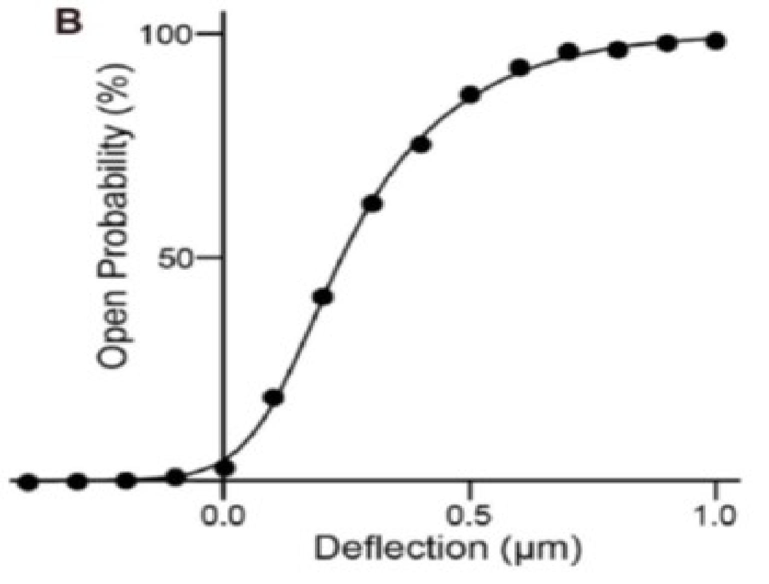
X0 – the displacement at which the channels are open 50% of the time, X0= 0.3 um; ***a*** - geometric factor (a>1)

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

(a= 0.1, X-X0= 500 nm, Δx= 2 nm)

Experiment

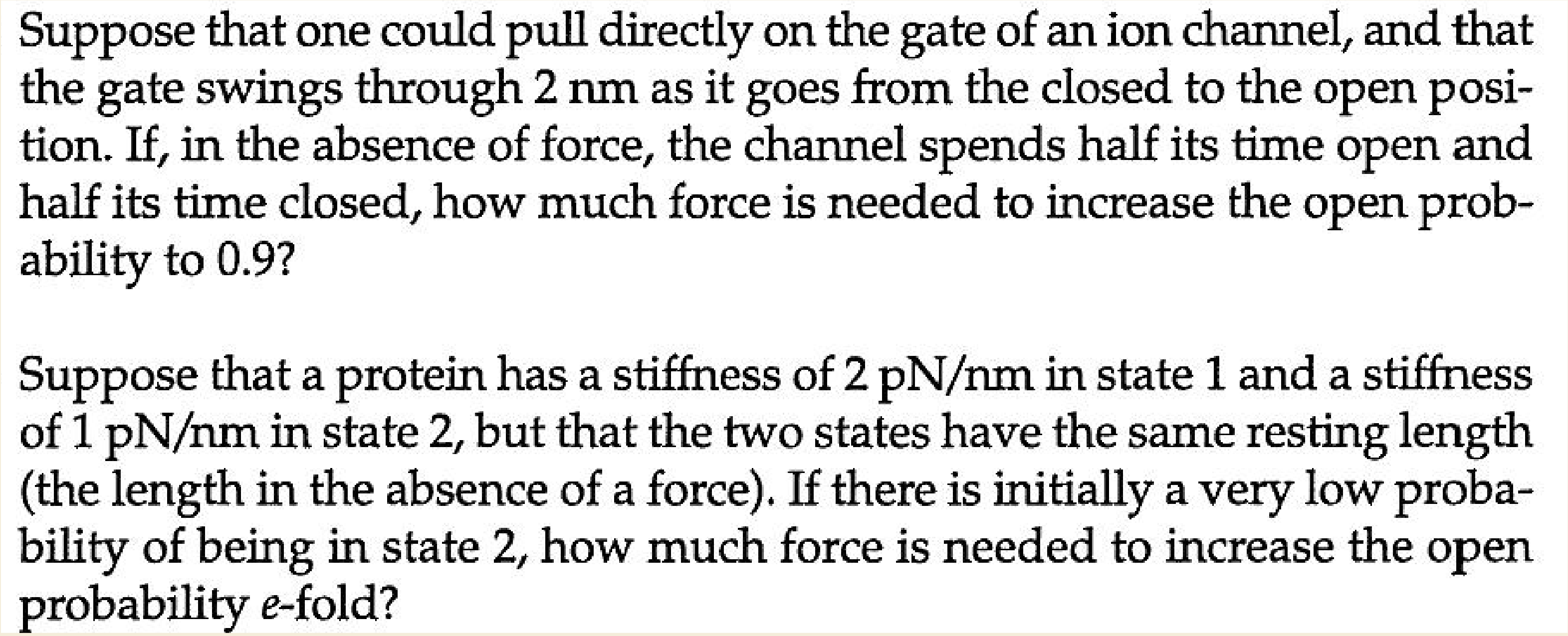




Problems

(force influencing the chemical equilibrium defined by the probabilty 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?

Rate theory of chemical reactions and the effect of force on the chemical rate constant

Forces also affect the rates of chemical reactions.

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

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

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}]. (Association and dissociation constants)

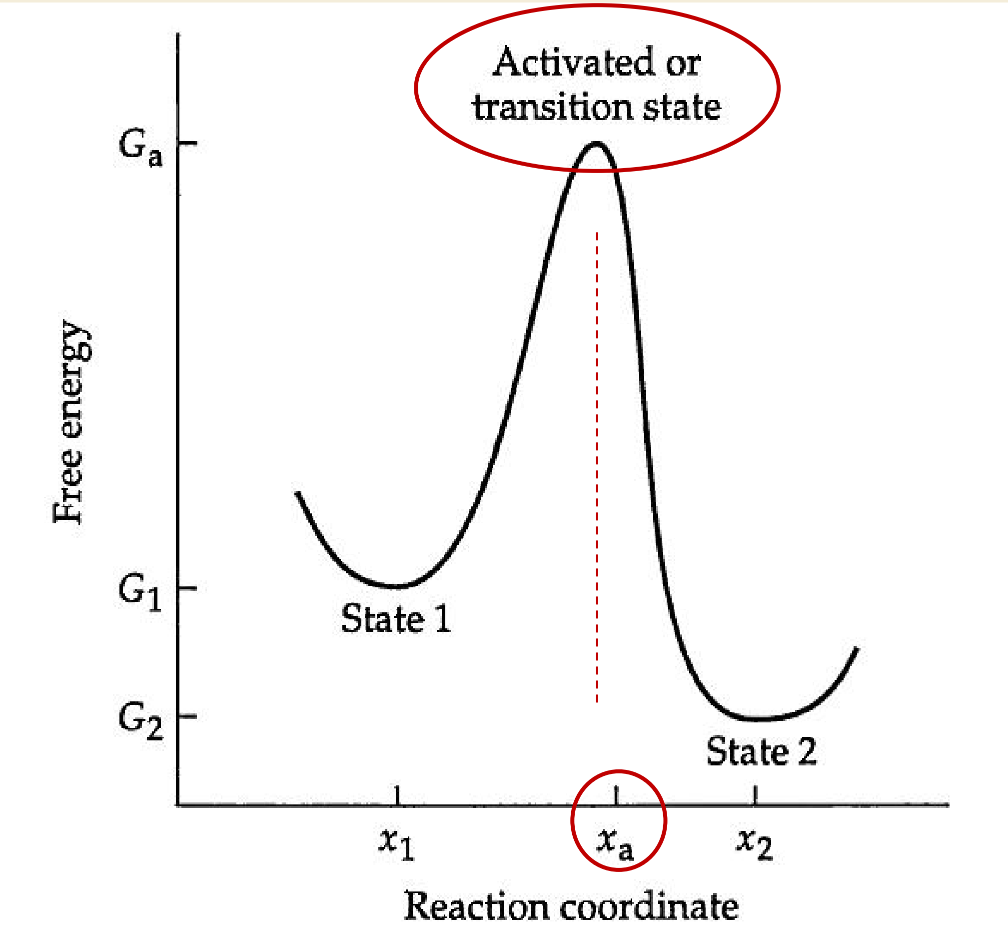
When the reaction reaches equilibrium, ($d[E_1]/dt=0$), and:

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

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.

Arrhenius theory / equation



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.

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

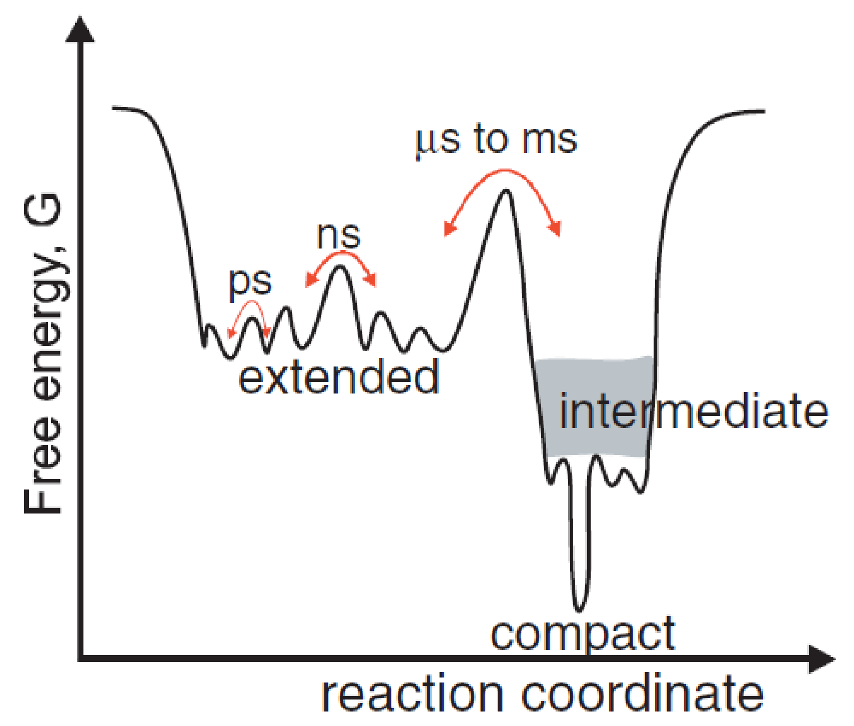
Arrhenius equation; *A*- frequency factor

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)

Eyring and Kramers theories

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

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

$$\Delta Ga_1 = G_{a1} \text{-} G_1$$

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,

$$E_1 \rightleftharpoons E_2$$

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, the reaction coordinate is the distance corresponding to unbinding.

Eyring theory

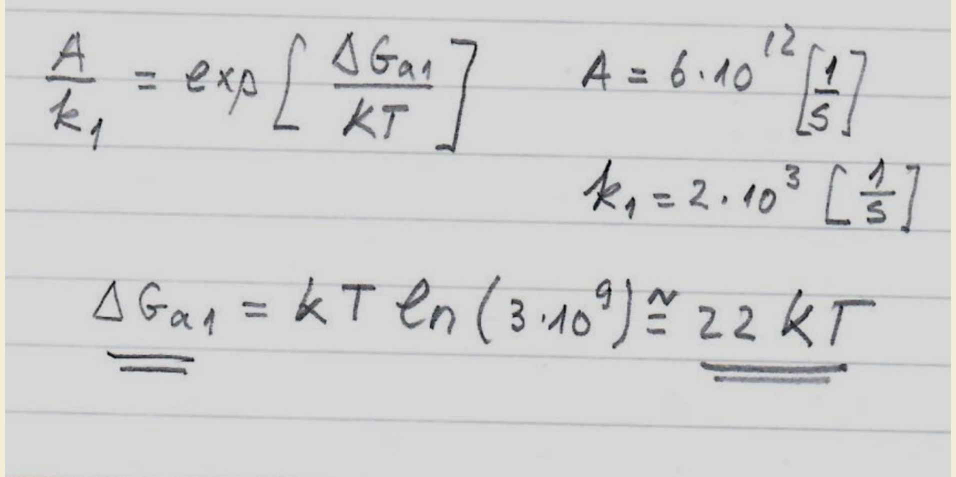
In the **Eyring rate theory,**

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

Therefore the frequency factor is considered ***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 : ΔGa_1 = 22 KT

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



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

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

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

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.

Kramers theory

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

$$\Delta Ga_1 = G_{a1} - G_1$$

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 *$\tau = \gamma/k$ (relaxation time)*

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.

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

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 τ= ɣ /k .

A ≈ KT/h $\quad$ $\mathbf{A_{Eyring}} \approx 10^{12}$ -- 10^{13} [s^{-1}]

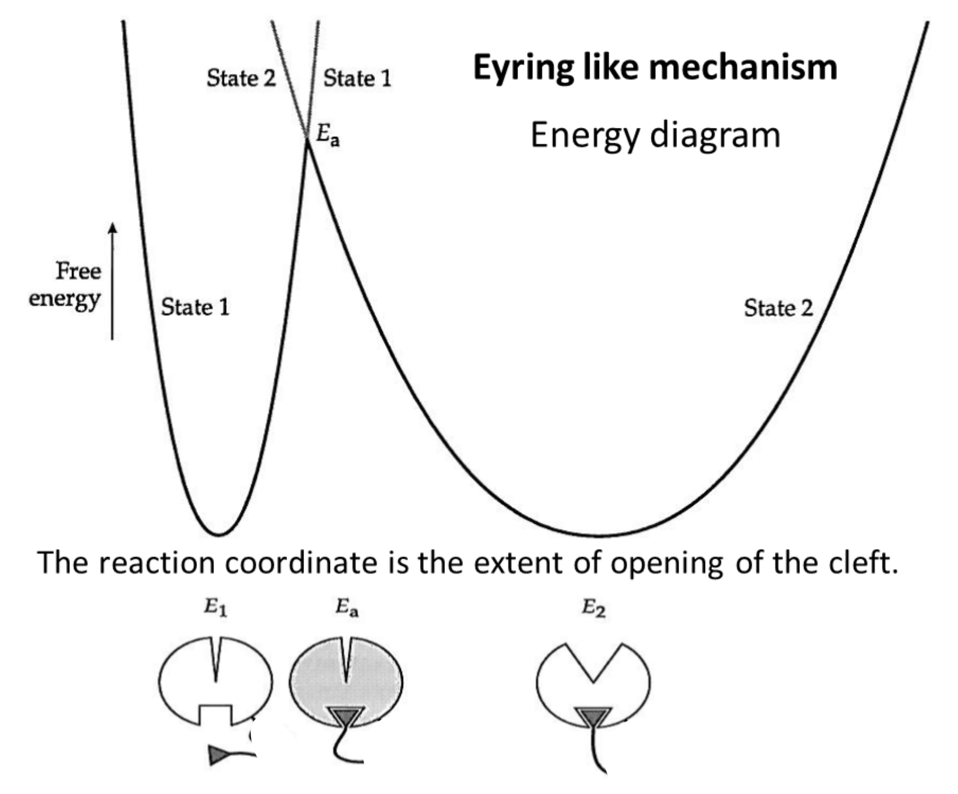
In the **Kramers** theory, the protein undergoes a global diffusion into the activated state.

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.

$A \approx \boldsymbol{1/\tau}\ \surd(\Delta \boldsymbol{Ga1/KT})$ $\quad$ with $\tau = ɣ/k$, $A_{Kramers} \approx 10^8 – 10^9$ [s^{-1}]

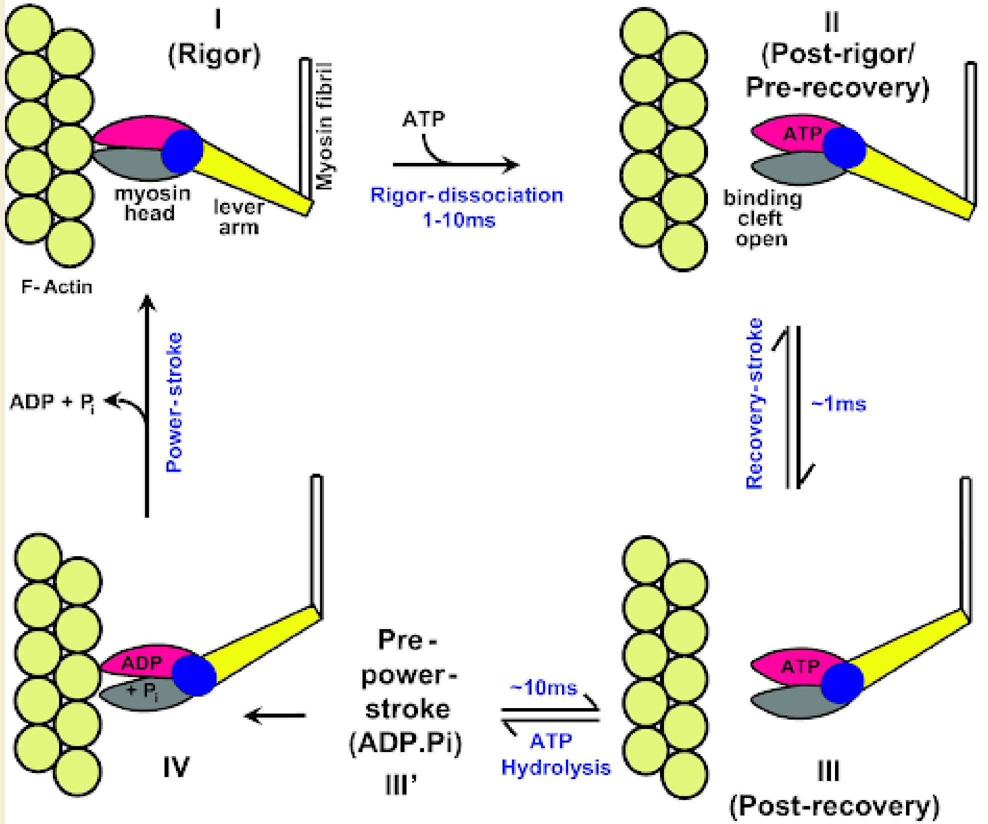
The model of the working stroke of myosin – assuming the Eyring like mechanism



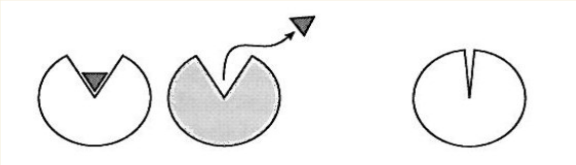
Eyring like mechanism

Energy diagram

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



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



Step III-IV : Phosphate rapidly dissocciates (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

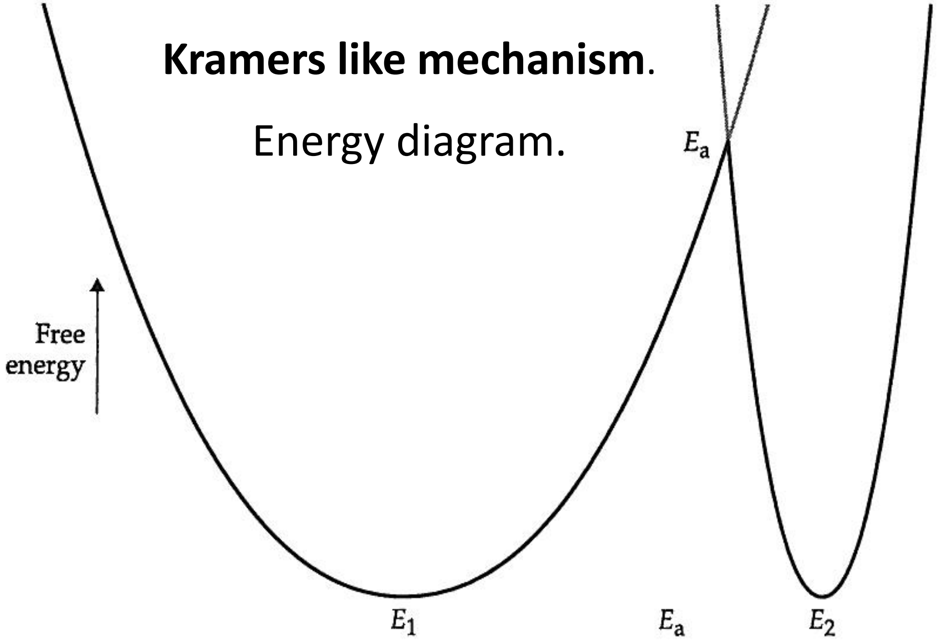
The principle for proteins is that global structural or 'physical' changes of proteins are much slower than local chemical changes, because structural changes are slowed by protein and solvent viscosity.

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

The thermal ratchet model of the working stroke of myosin – assuming the Kramers like mechanism

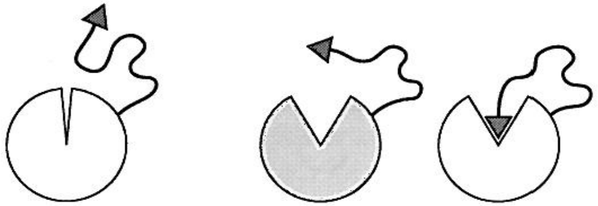
Kramers like mechanism.

Energy diagram.



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 γ= 60 pN s/m, and the relaxation time: $\tau = \gamma/k$= 15 ns

It takes about 2 s to pick up 20 KT of energy by a purely diffusive process:

$$k_1 = A\,exp\left[-\frac{\Delta G_{a1}}{KT}\right] \qquad \boldsymbol{A \approx \frac{1}{\tau}\sqrt{\frac{\Delta Ga_1}{KT}}}$$

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 1.5 ms.

More details on the mechanisms in force generation in motor proteins – Lecture Motor proteins

The model of the working stroke of myosin – assuming the Kramers like mechanism

In the Kramers view, 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 the way to the final state, which is then locked in by a subsequent chemical change .

This extreme case is called a **thermal ratchet** mechanism on account of the prominent role played by diffusion in reaching the transition state.

If the forward process is purely diffusive, then the reverse is Eyring like. However, even in the Eyring mechanism the activated state is also reached by a thermal fluctuation, which is more localized.

Rate theory of chemical reactions and the effect of force on the chemical rate constant

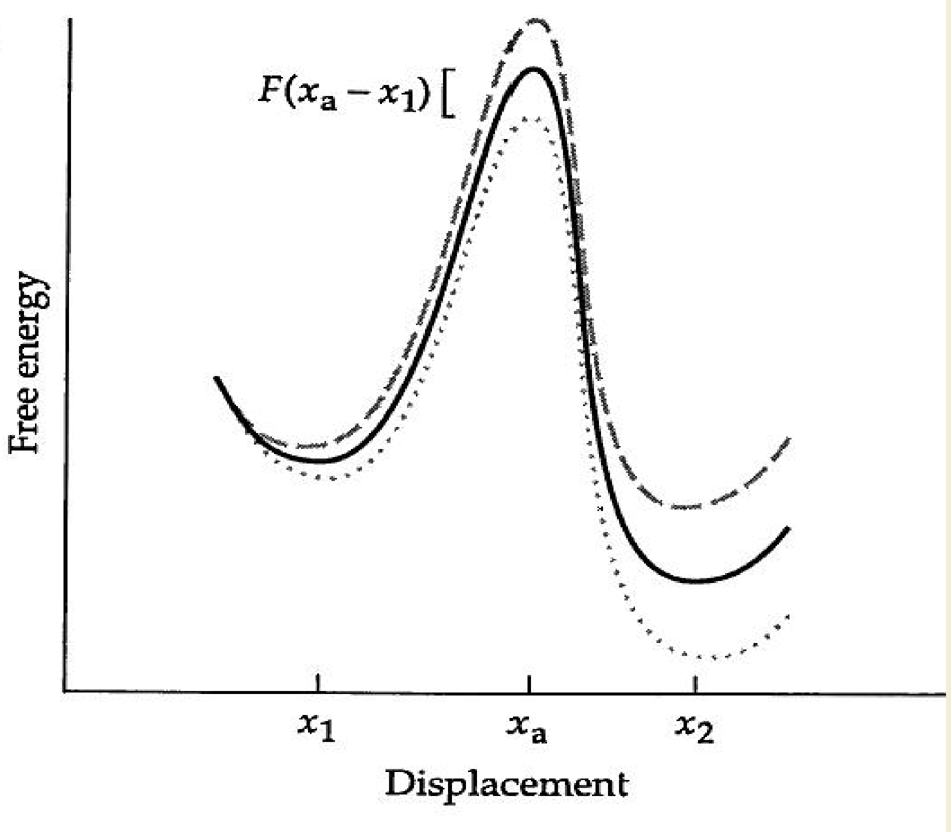
If the state transitions $E_1 \rightarrow E_a \rightarrow E_2$ are associated with displacements x1, xa, and x2 in the direction of the force, F, then the energies of the states will be decreased by Fx1, Fxa, and Fx2, respectively.

This implies:

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

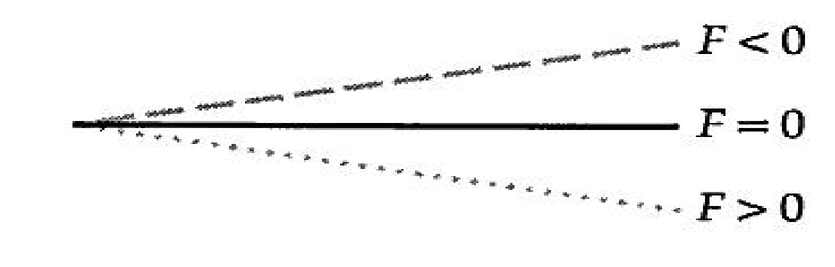
where $\Delta G_{a1} = G_{a1} - G1$ and $\Delta x_{a1} = X_{a1} - x_1$.

An analogous expression holds for k_{-1}.



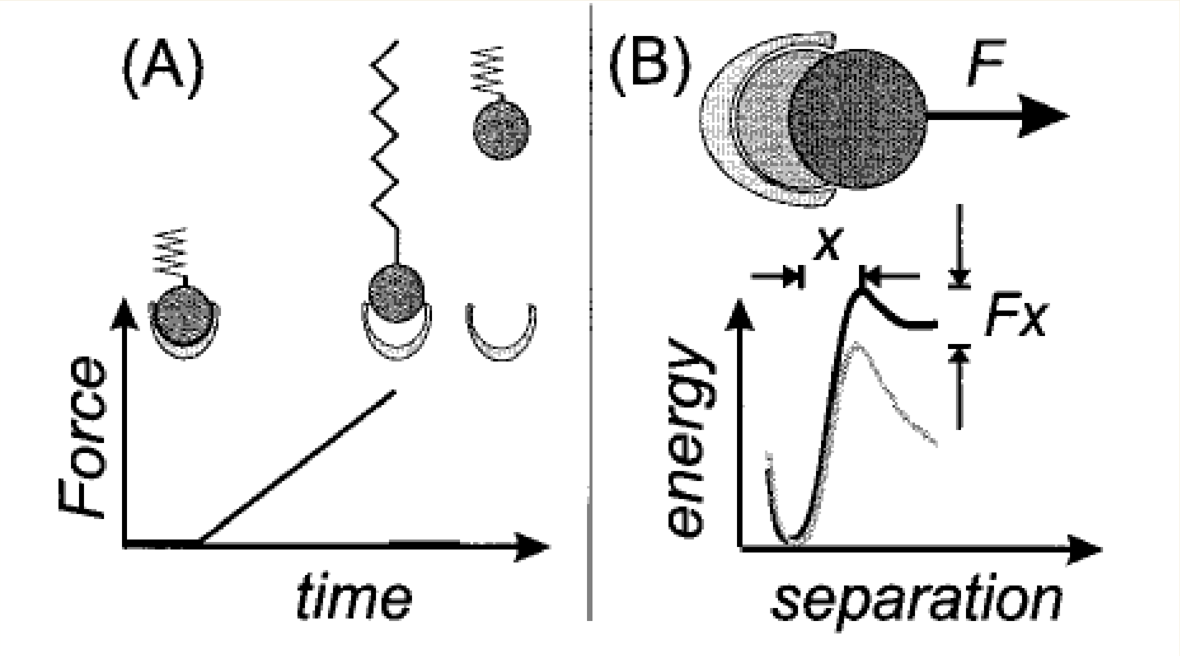
The effect of force on the reaction rates:

tilting the free energy diagram.



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

Model and design experiment – single molecule vs bulk



F= r t with r – loading rate

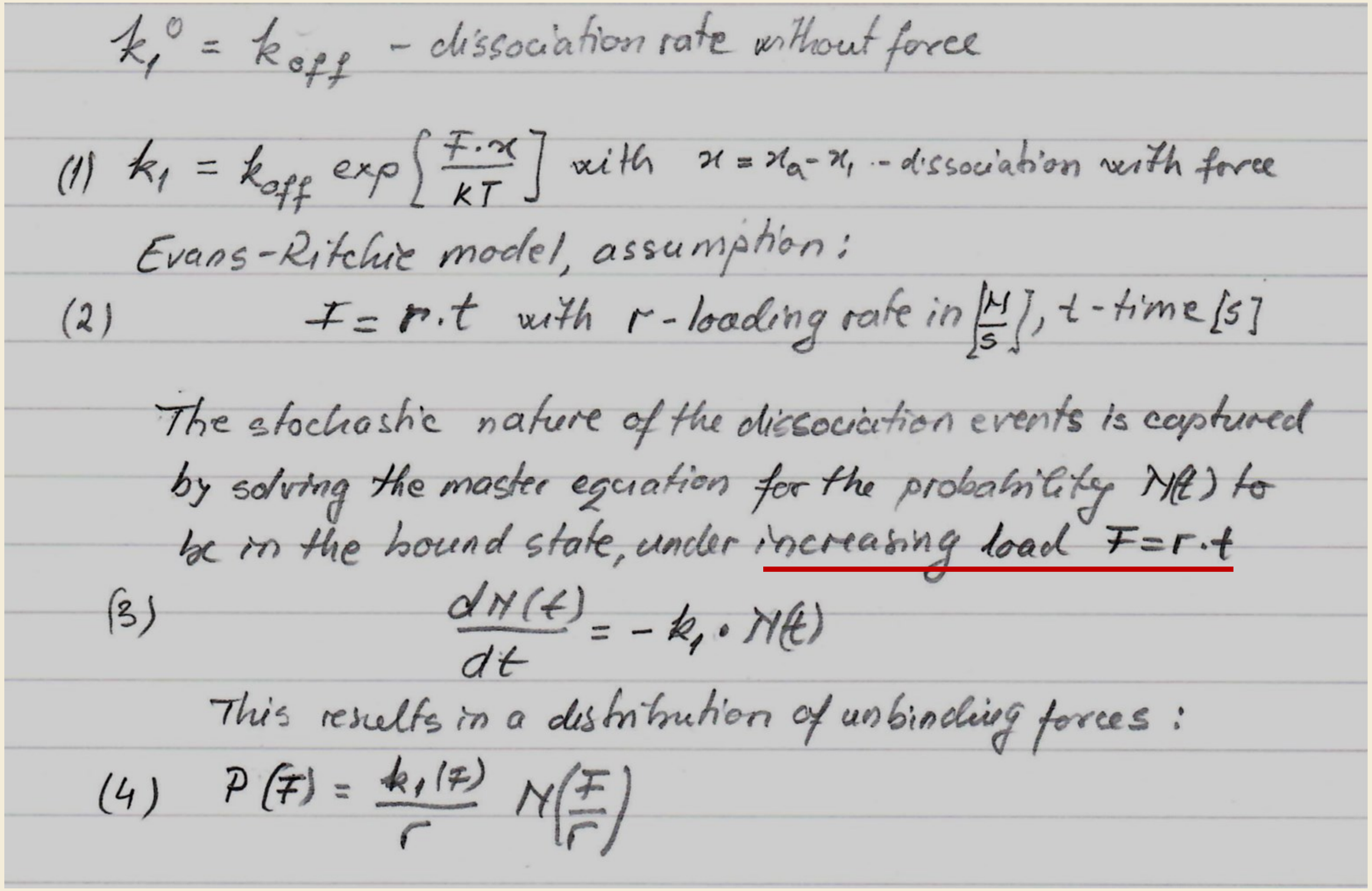
(A) Direct observation of the dissociation under a mechanical force. The force on a single complex increases until it dissociates. The dissociation is monitored by an abrupt relaxation of the macroscopic spring of a force probe.

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

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

$$k_1^0 = \frac{KT}{h}\, exp\left[-\frac{\Delta Ga_1}{KT}\right]$$



$k_1^0 = k_{off}$ - 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} (k_1^0)

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.

Evans, Probing the relation between force – lifetime – and chemistry in single molecular bonds, Annu. Rev. Biophys Niomol Struct. 2001. 30:105-27

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

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

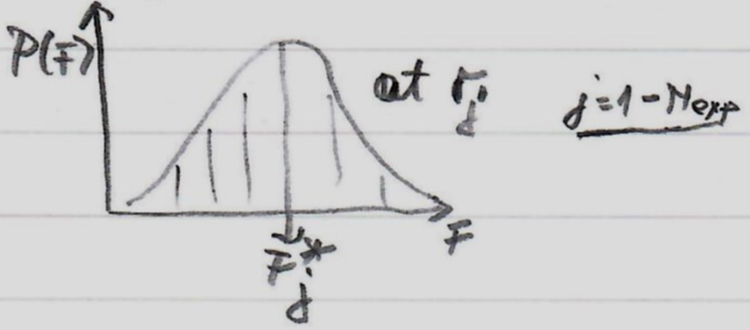
(6) The most probable unbinding/dissociation force

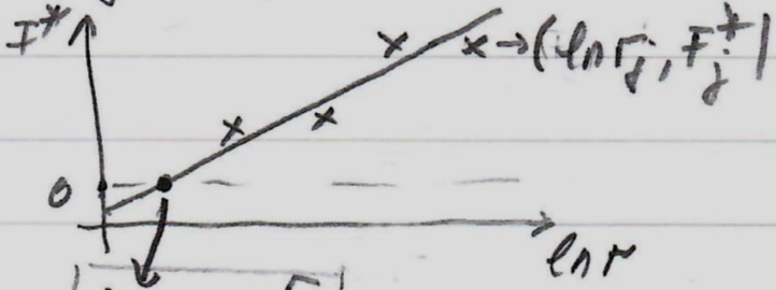
$$F^* \doteq \frac{kT}{x}\ln\left(\frac{x}{kT}\frac{r}{k_{off}}\right) \qquad \frac{kT}{x}=\varrho$$

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

F^* linear with $\ln r$

F_j^* measured for ~~rate~~ loading rate r_j, $j = 1 - N_{experiments}$





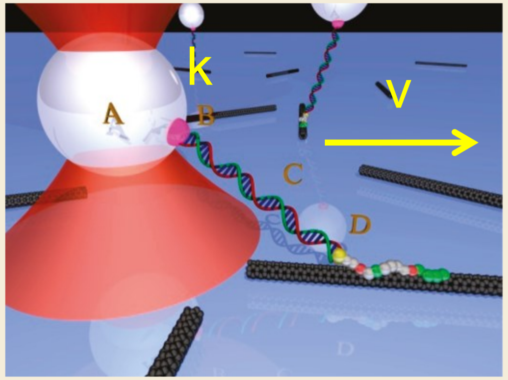
$$\boxed{k_{off} = \frac{r_e}{\varrho^e}}$$

Linear fit + Extrapolation

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

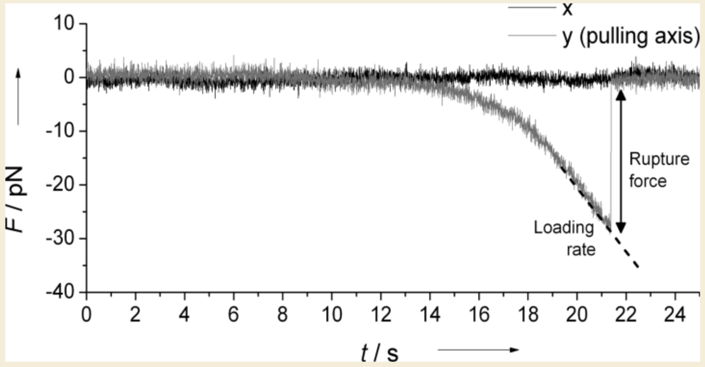
Use the force to measure unbinding forces and dissociation rate k_{off} of Ligand to Receptor

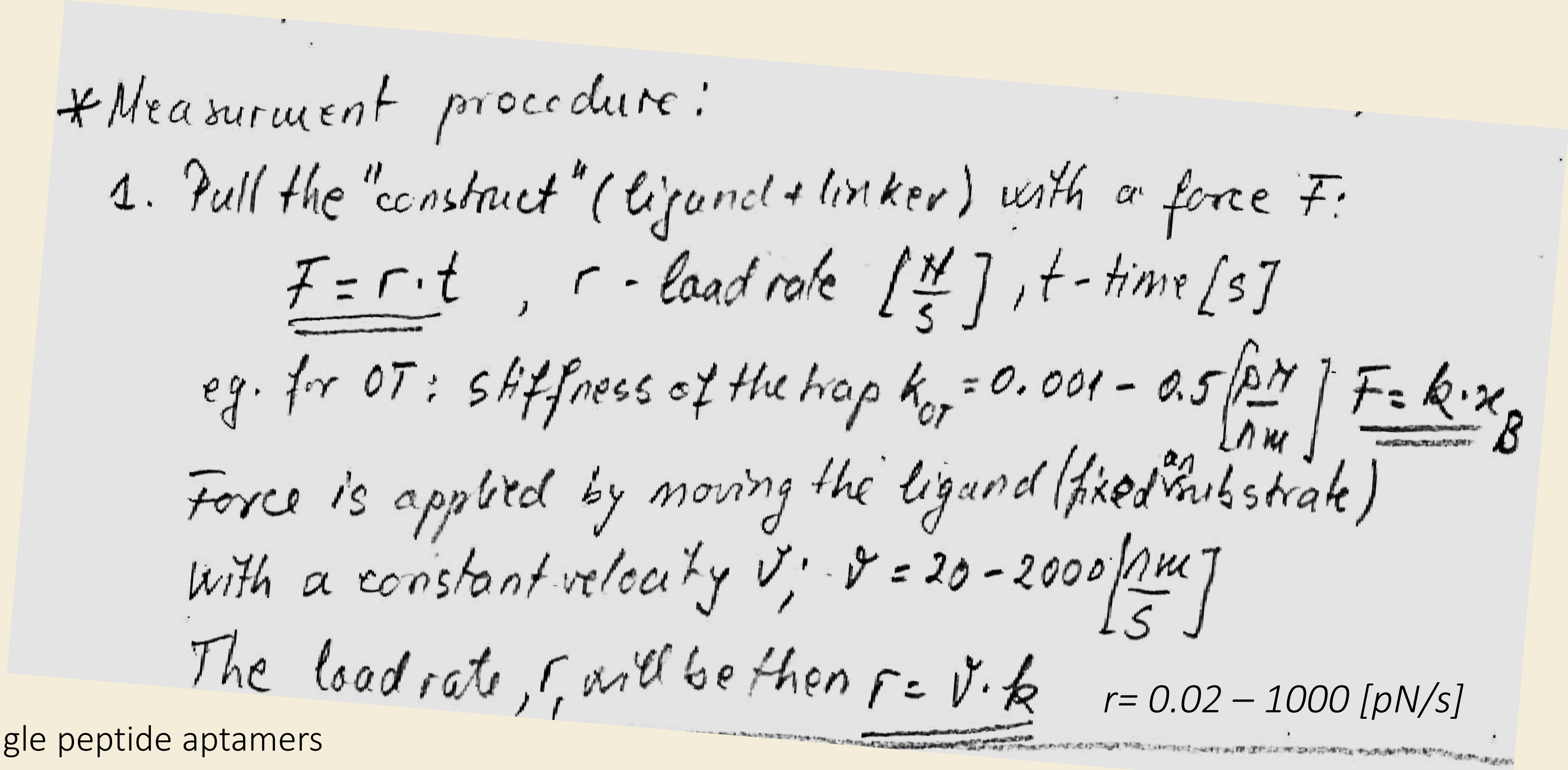
How it works in practice / experimentally ?



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 exert 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:

$\underline{F = r \cdot t}$, r - load rate $[\frac{N}{s}]$, t - time [s]

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

Force is applied by moving the ligand (fixed an substrate)

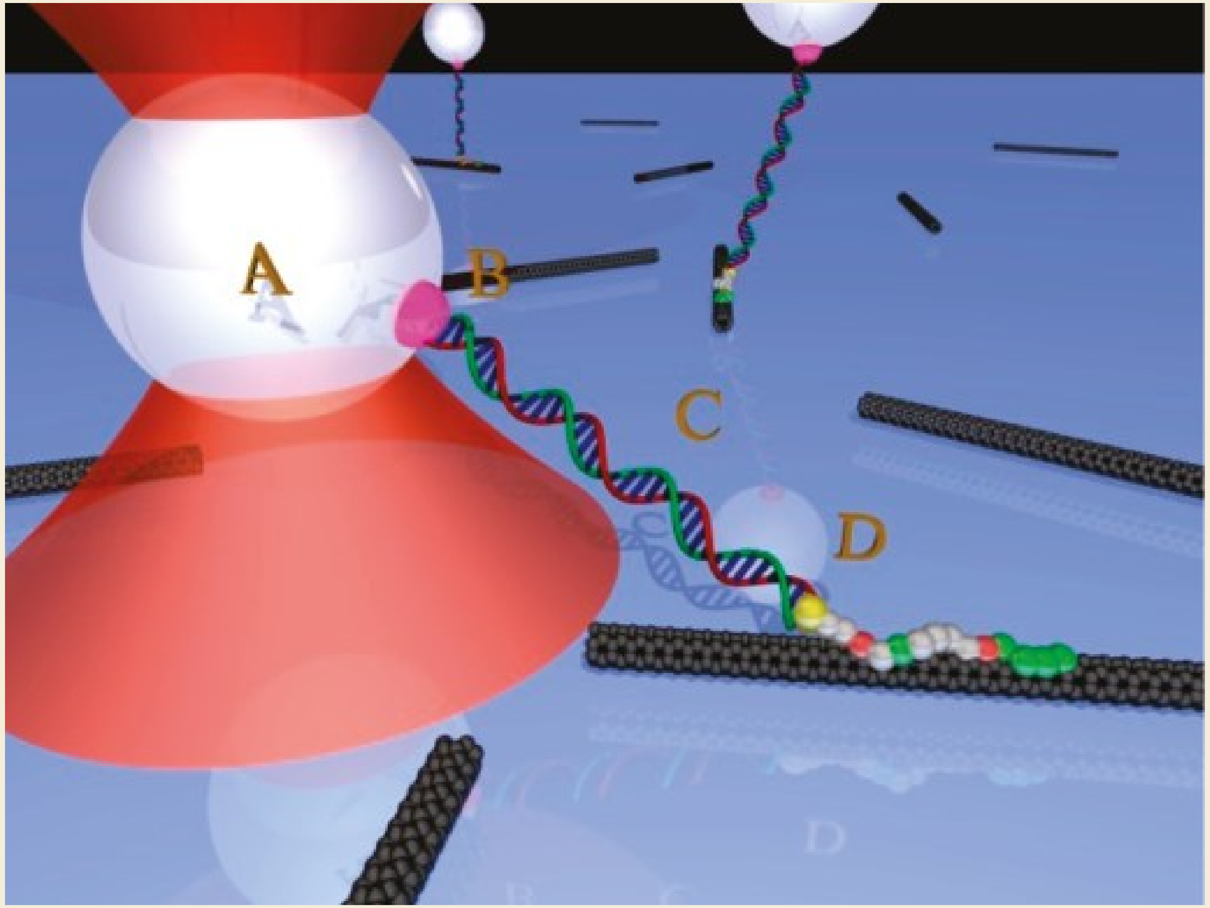
with a constant velocity v; $v = 20 - 2000 [\frac{nm}{s}]$

The load rate, r, will be then $\underline{r = v \cdot k}$

r= 0.02 – 1000 [pN/s]

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

How the experiment is developed



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.

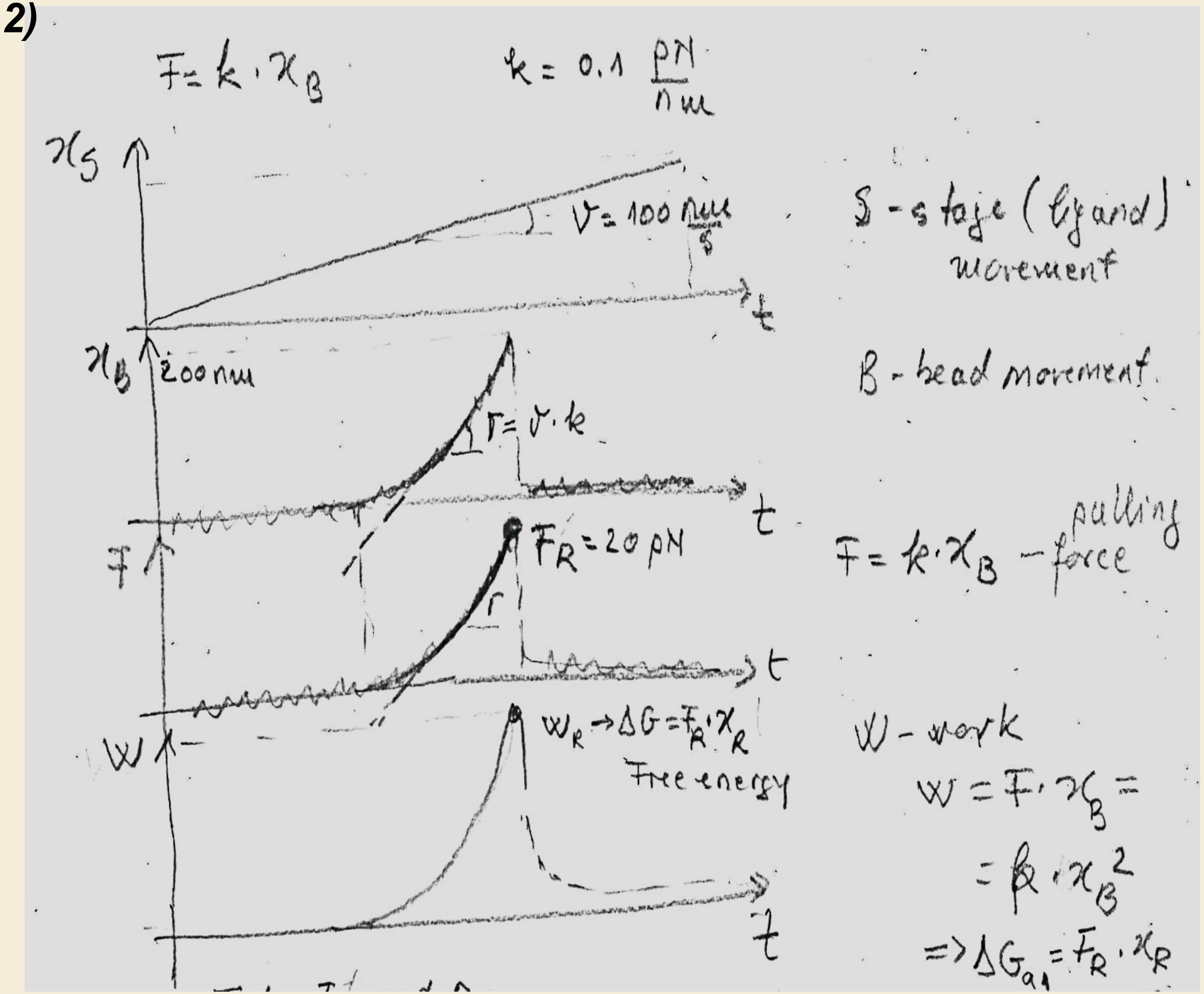
Peptide aptamers typically contain 8-20 amino-acids and bind materials or biomolecules.

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 |J. Phys. Chem. A 2011

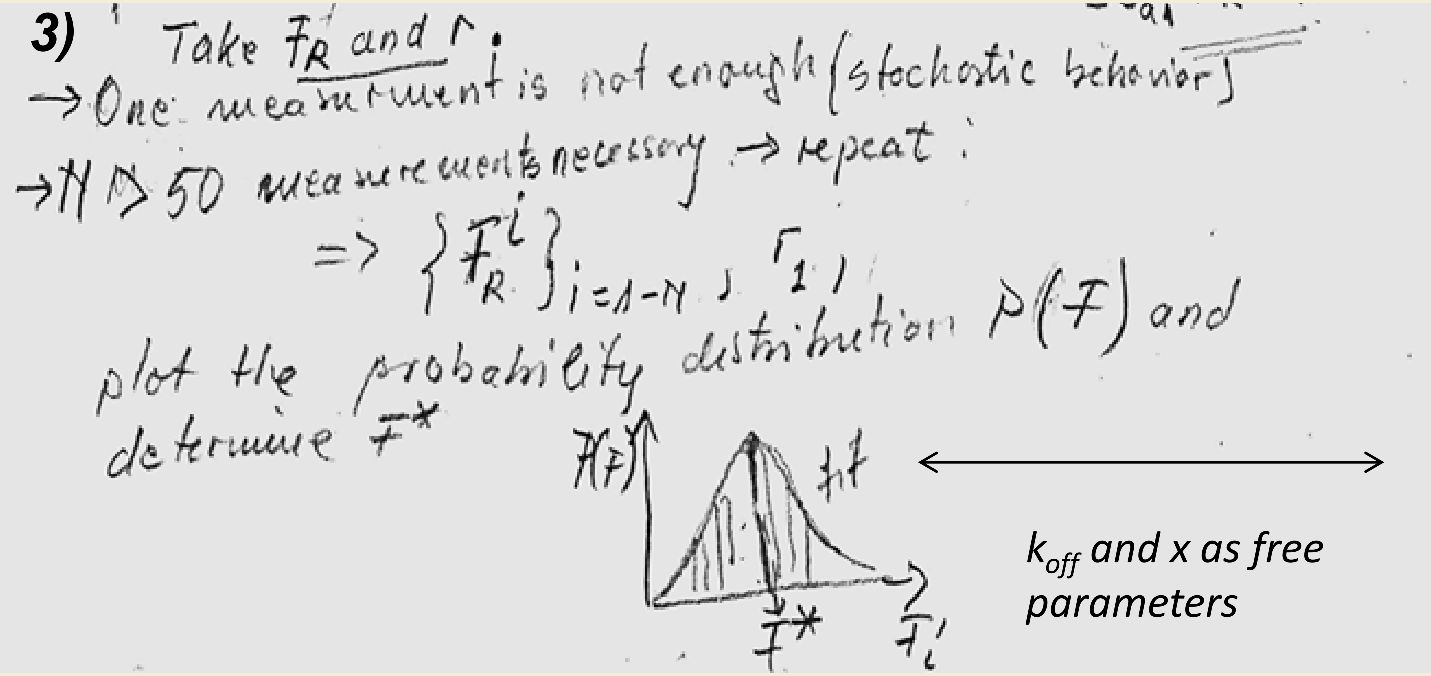
2)



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)

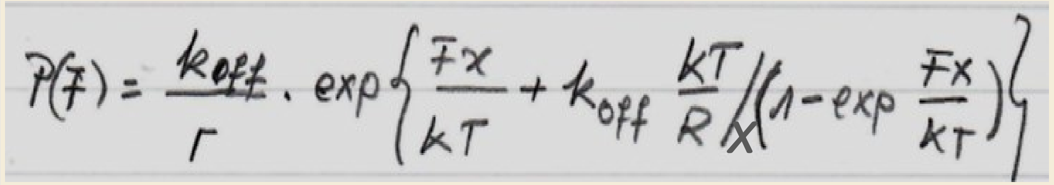
→ $N > 50$ measurements necessary → repeat:

$$\Rightarrow \{F_R^i\}_{i=1-N}, \; r_1,$$

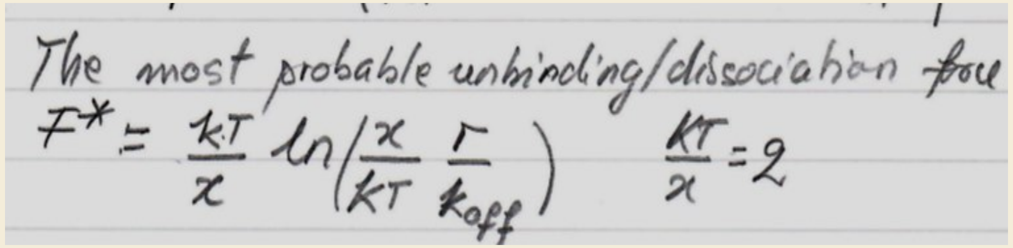
plot the probability distribution $P(F)$ and determine F^*

↔

k_{off} and x as free parameters



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



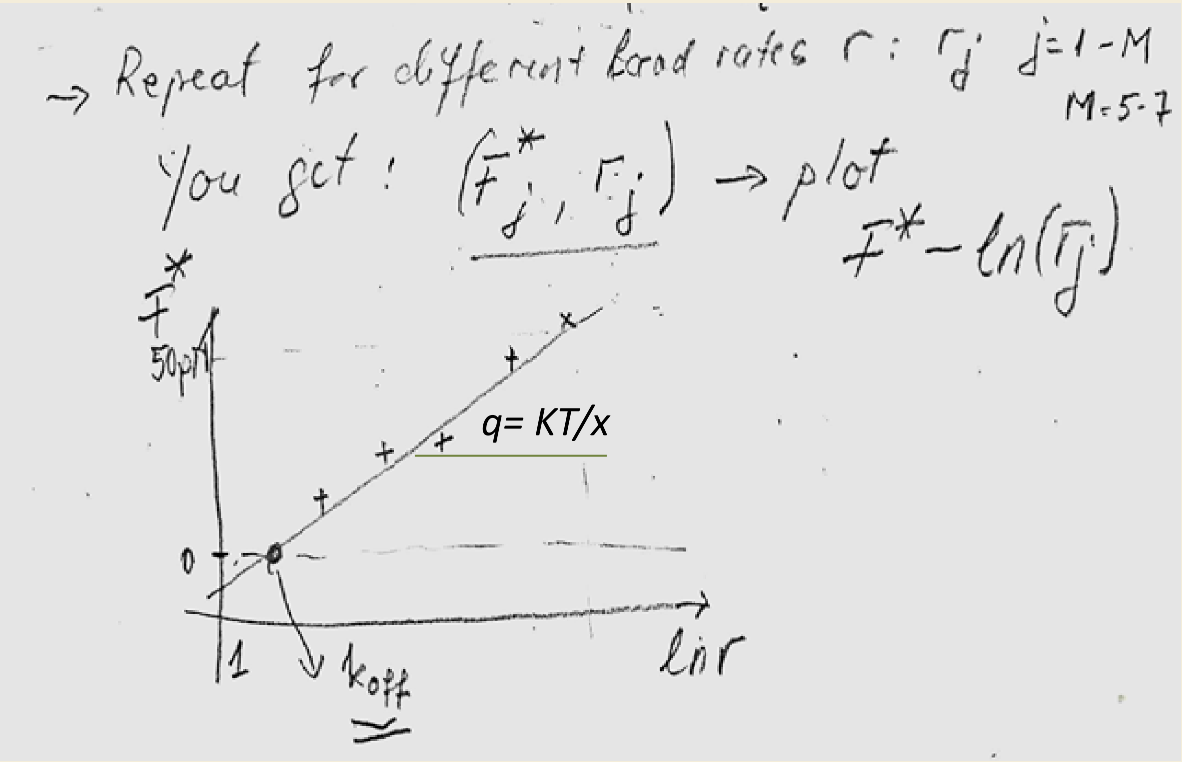
The most probable unbinding/dissociation force

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

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

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

4)

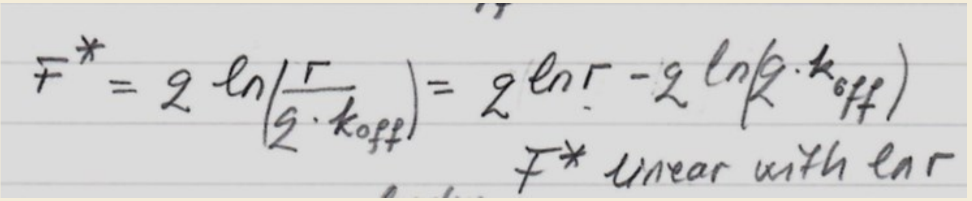


→ Repeat for different load rates r: r_j $j=1-M$

$M=5-7$

You get: $\underline{(F^*_j, r_j)}$ → plot

$F^* - \ln(r_j)$



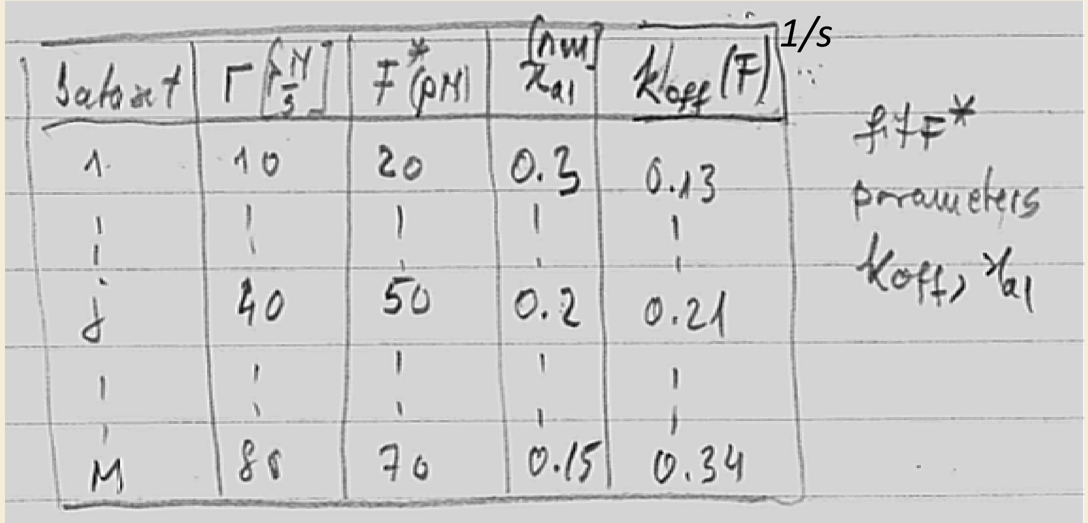
$$F^* = q \ln\left(\frac{r}{q \cdot k_{off}}\right) = q \ln r - q \ln(q \cdot k_{off})$$

F* linear with ln r

The slope is q= KT/x

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

r_0 – extracted load rate r for F=0*

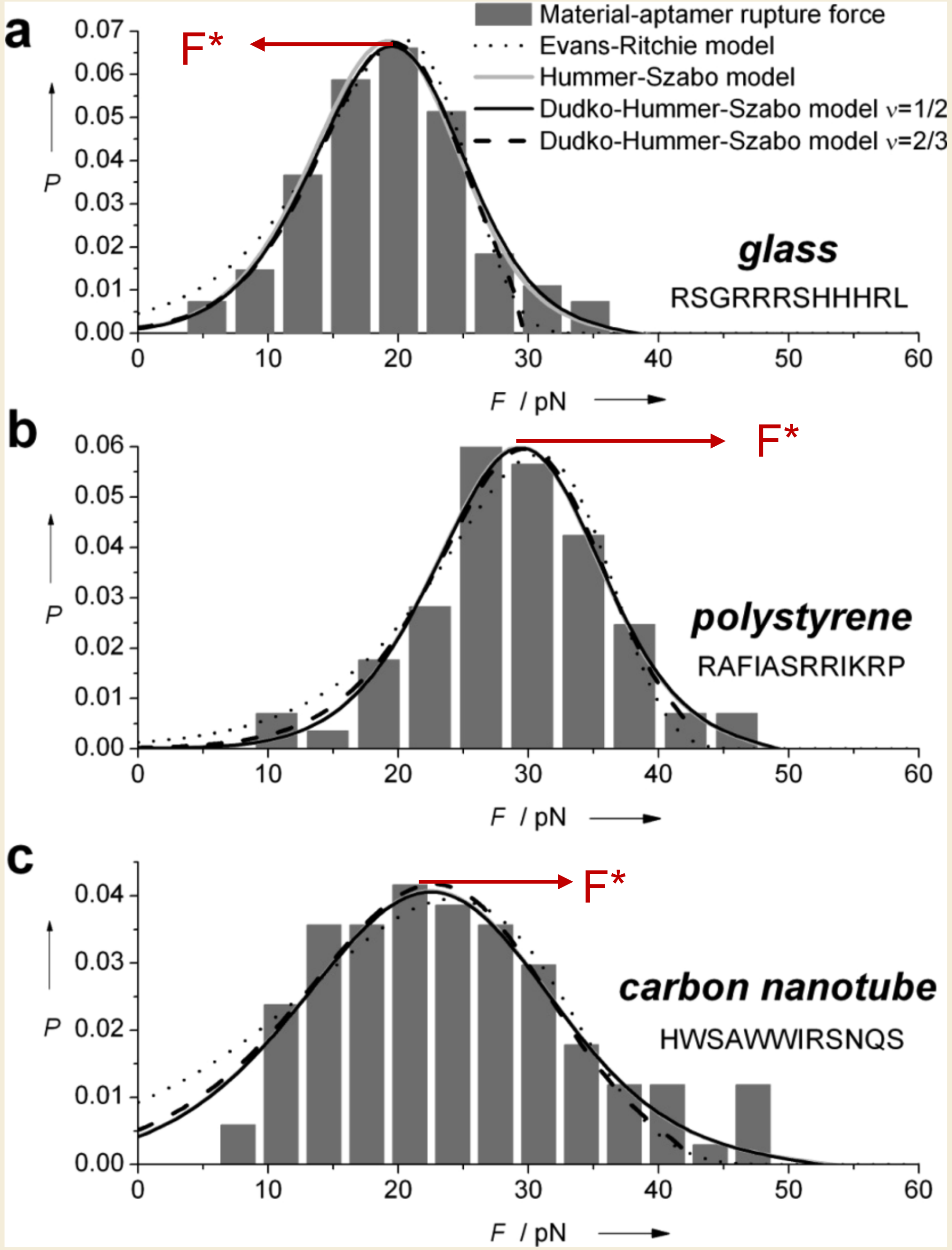


Dataset	r [pN/s]	F* (pN)	x_{a1} [nm]	k_{off} (F) 1/s
1	10	20	0.3	0.13
⋮	⋮	⋮	⋮	⋮
j	40	50	0.2	0.21
⋮	⋮	⋮	⋮	⋮
M	88	70	0.15	0.34

fit F*
parameters
k_{off}, x_{a1}

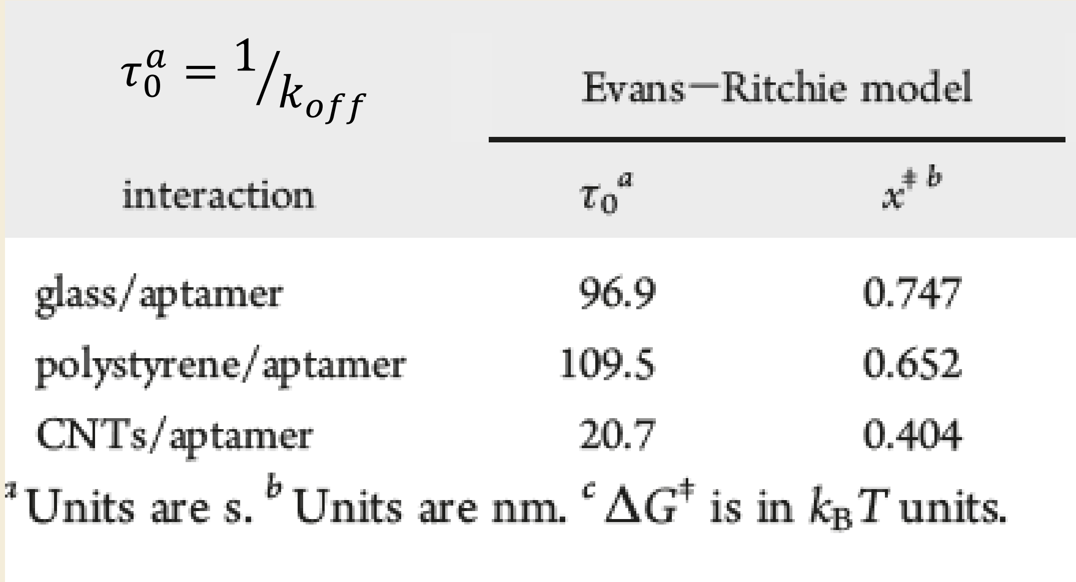
$$k_{off}(F^*) = k_{off} \cdot \exp(F^* x / KT)$$

model prediction



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

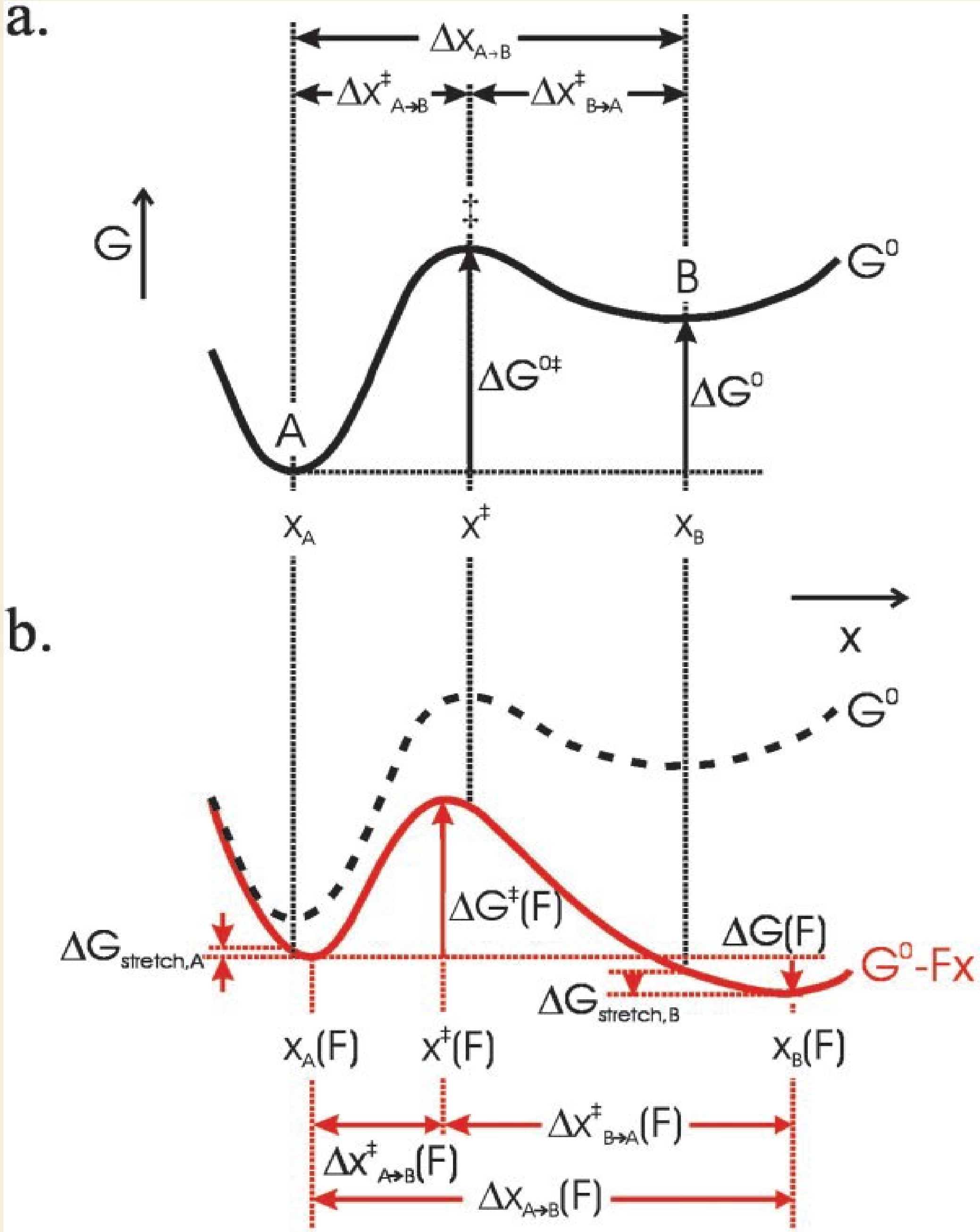
(a) glass,
(b) polystyrene, and
(c) carbon nanotubes



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

	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_BT 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 ‡ 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 $\Delta G_{\text{stretch A, B}}$

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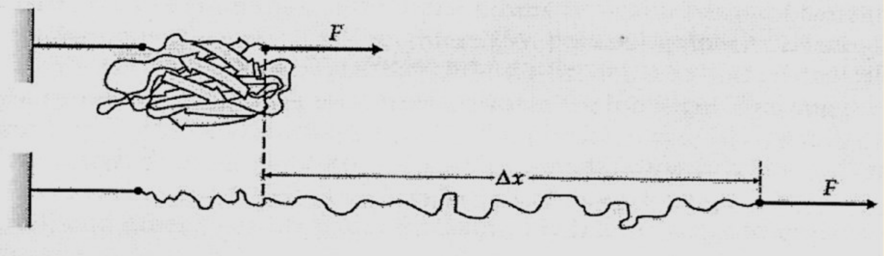
Carlos Bustamante et all Review

Unfolding rate of a single protein domain – Immunoglobulin IG27

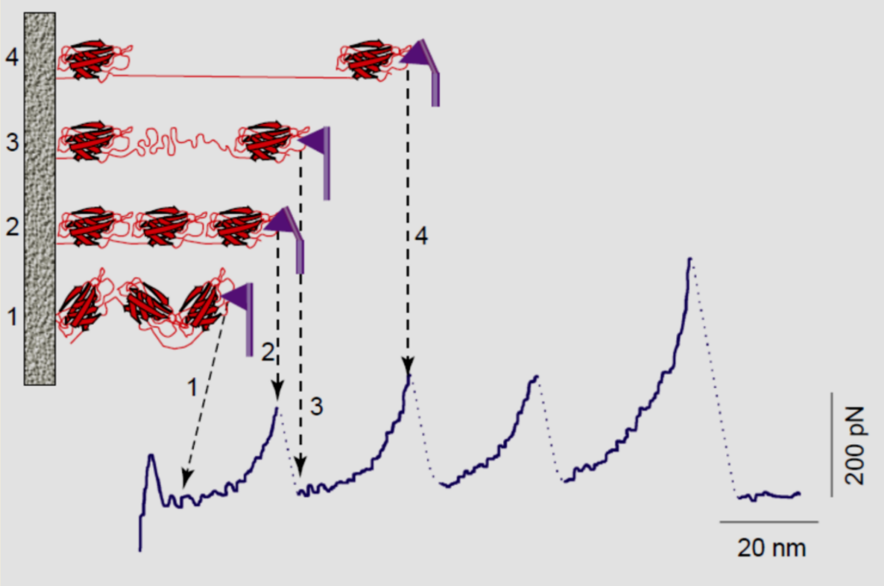
Carrion-Vasquez et al, PNAS 96:3494, 1999

Mechanical and chemical unfolding of a single protein: a comparison

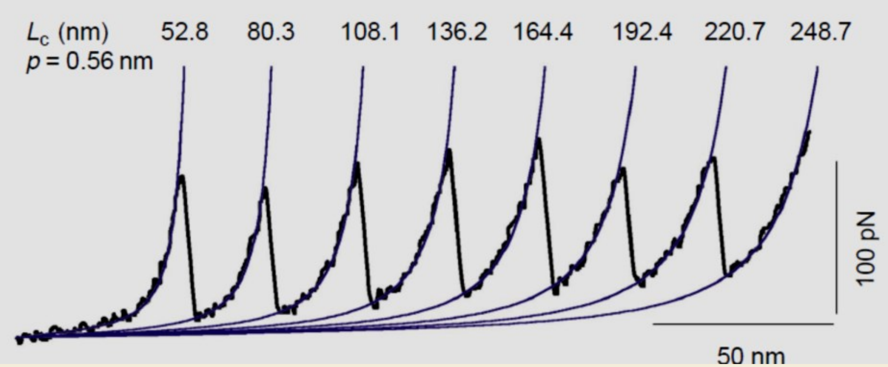
AFM was used to reversibly unfold immunoglobulin modules (IG27) domain. And measure the unfolding rate, which was compared with that obtained from chemical unfolding using a denaturant reaction.



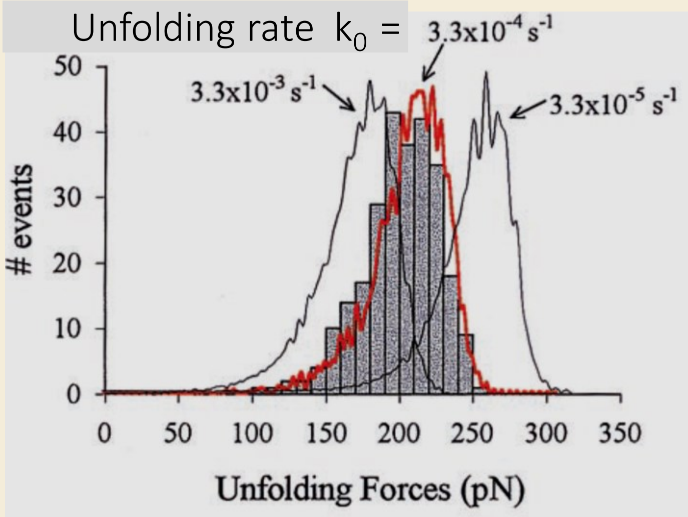
Experiment principle



Force-extension relationship for recombinant poly(I27)

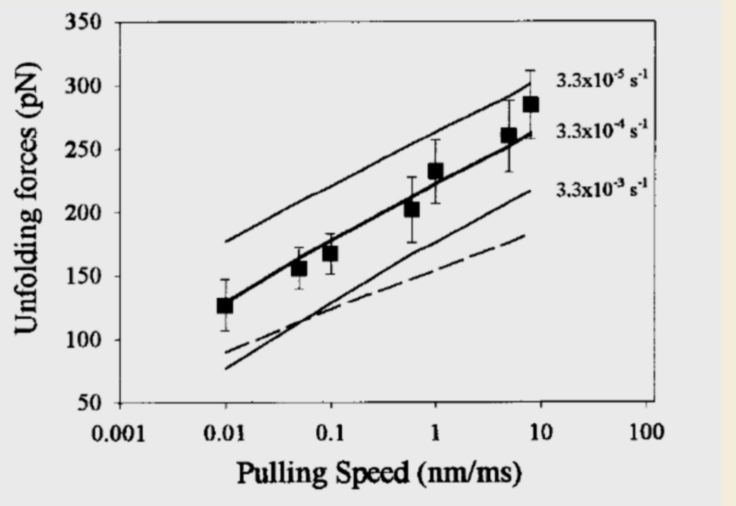


Unfolding force frequency histogram



Unfolding rate k_0 =

Unfolding force vs pulling speed



WLC model (Worm Like Chain)

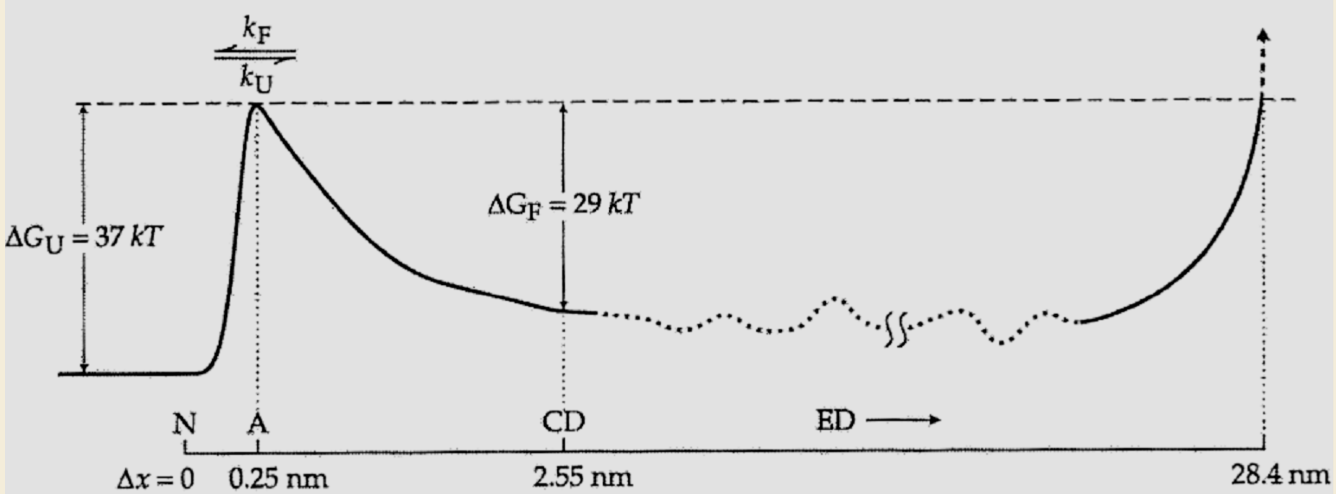
$$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{2} - \frac{1}{4} + \frac{x}{L_c}\right]$$

unfolding distance Δx=0.25 nm,
pulling rate v = 0.6 nm/ms.

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

Unfolding rate of a single protein domain

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



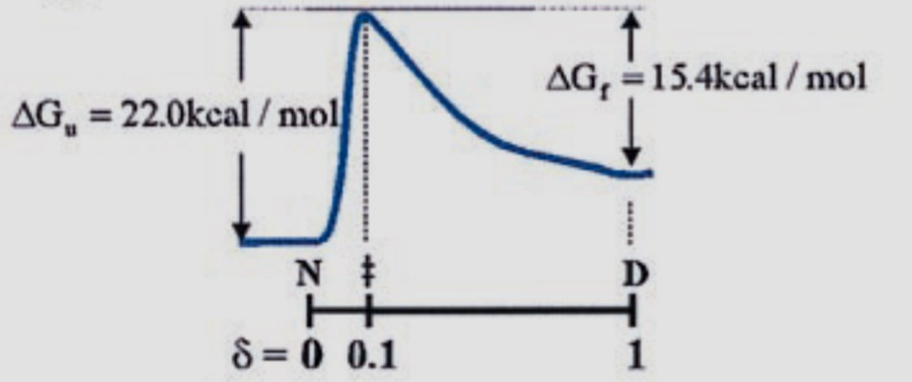
N = native state, A = activated state, CD = compact disordered state, ED = extended state

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.

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

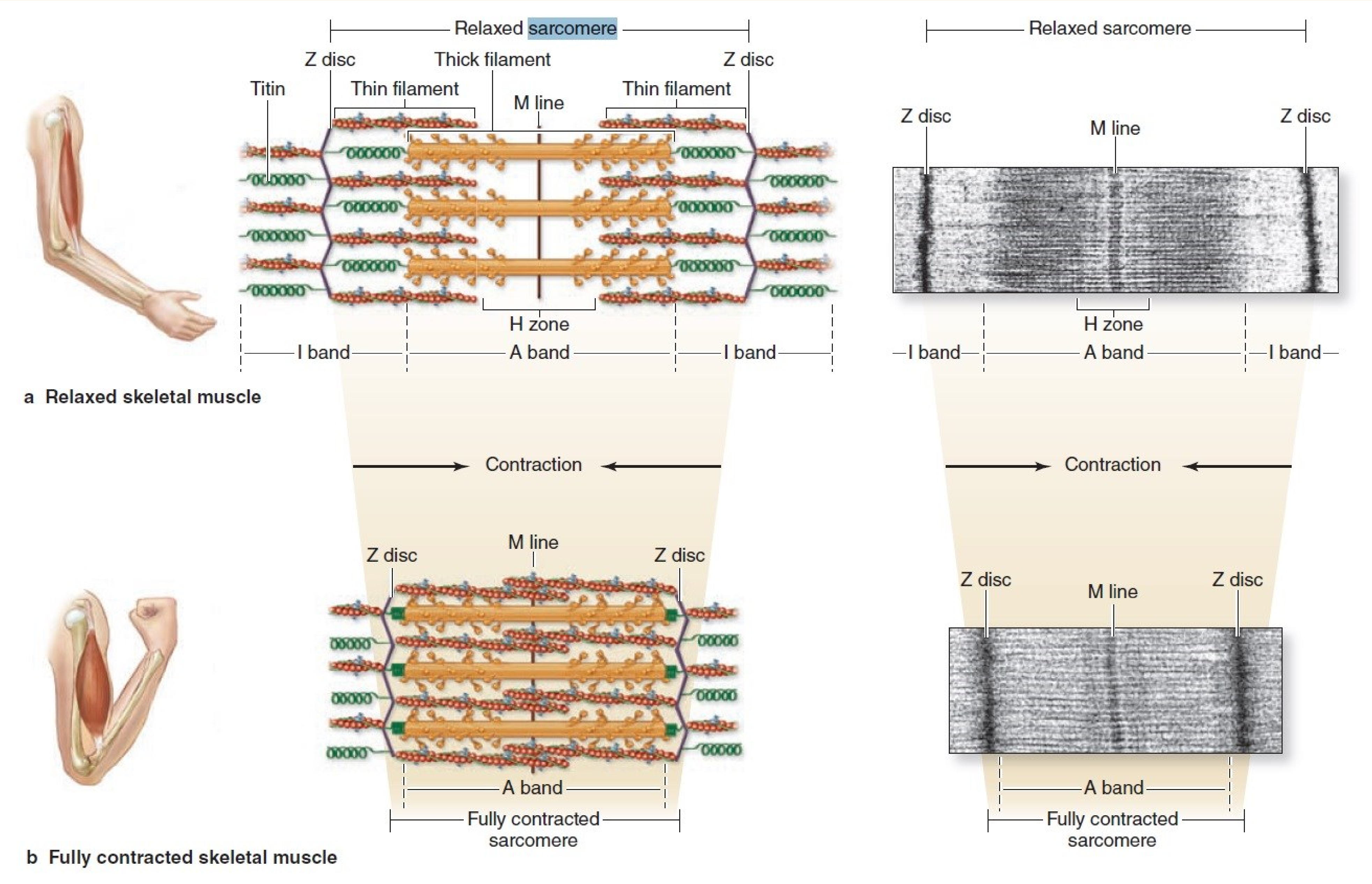


1 kcal/mol → ~1.7 KT / molecule

Carrion-Vasquez et al, PNAS 96:3494, 1999 Mechanical and chemical unfolding of a single protein a comparison

Sarcomere shortening during skeletal muscle contraction.

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



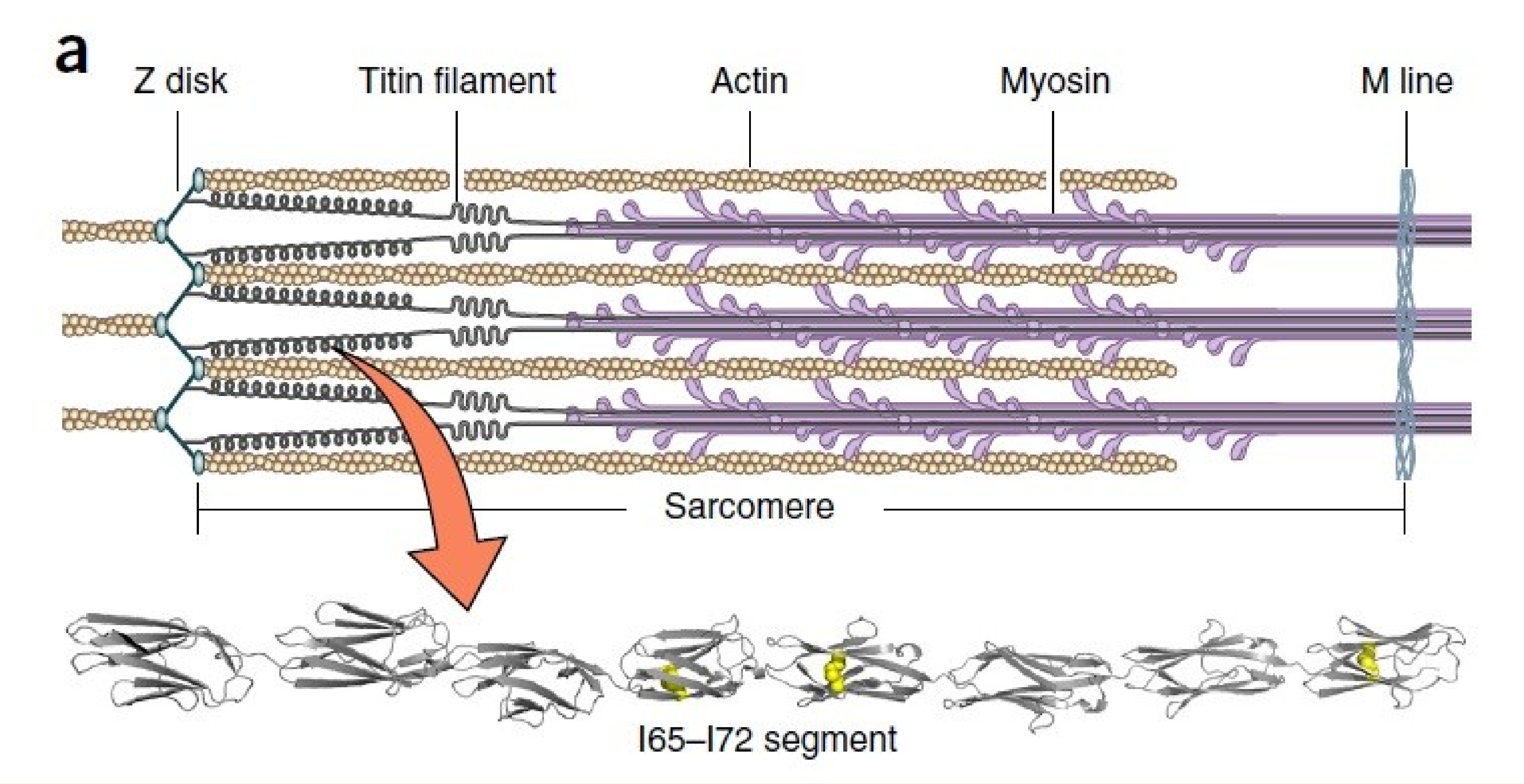
EM images

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 first single proetin unfolding was studied with 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 um).

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.

↓

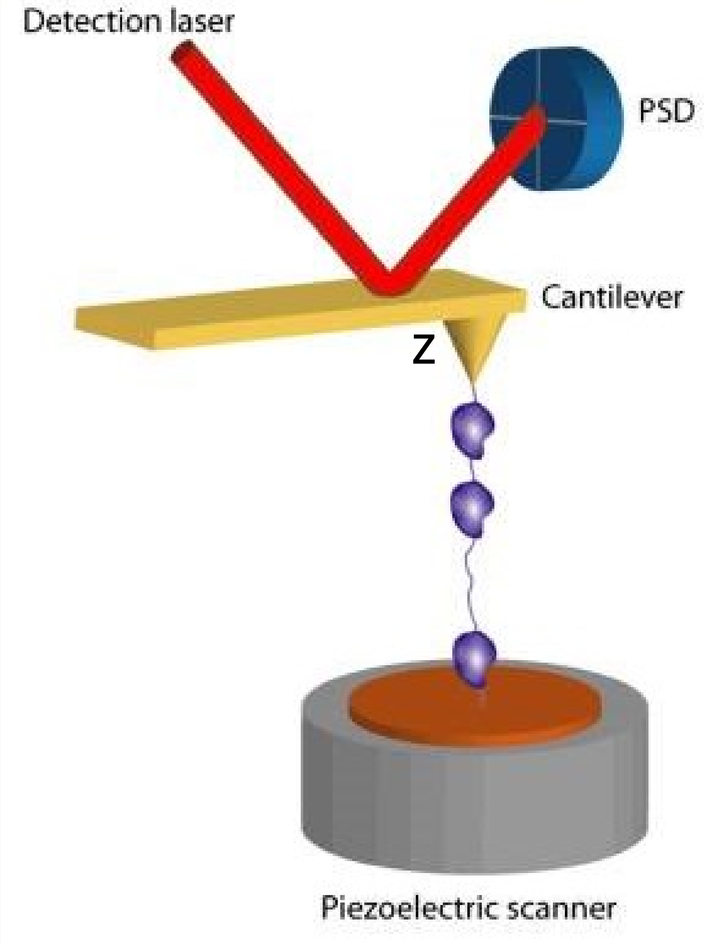
Rief M, Gautel M, Oesterhelt F, Fernandez JM, Gaub HE. 1997.
Reversible unfolding of individual titin immunoglobulin domains by AFM.
Science 276:1109–12

!! Published in the same issue of Science

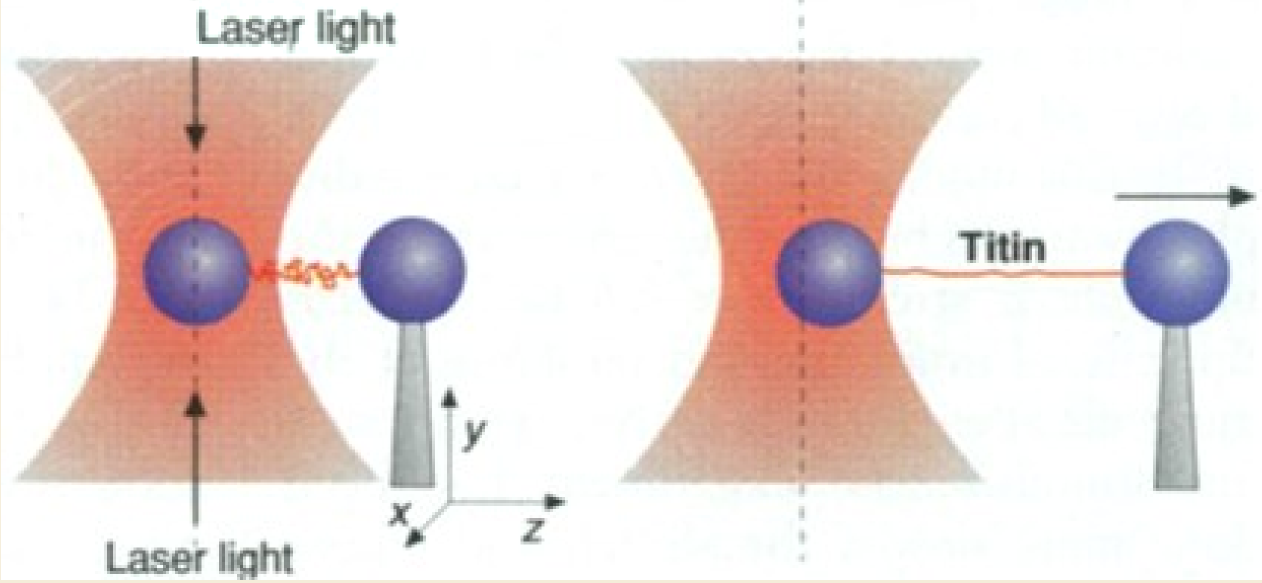
M. S. Z. Kellermayer, S. B. Smith, H. L. Granzier, C. Bustamante,
Folding-Unfolding Transitions in Single Titin Molecules Characterized with Laser Tweezers, Science 276, 1112–1116 (1997).

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

$k_{AFM} >> k_{OT}$

$\downarrow$

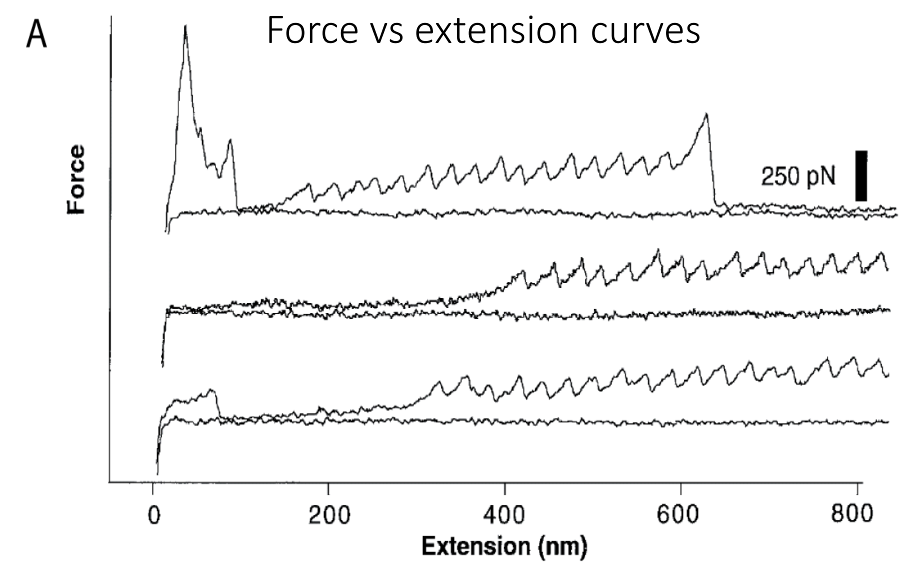
Force resolution: AFM < OT

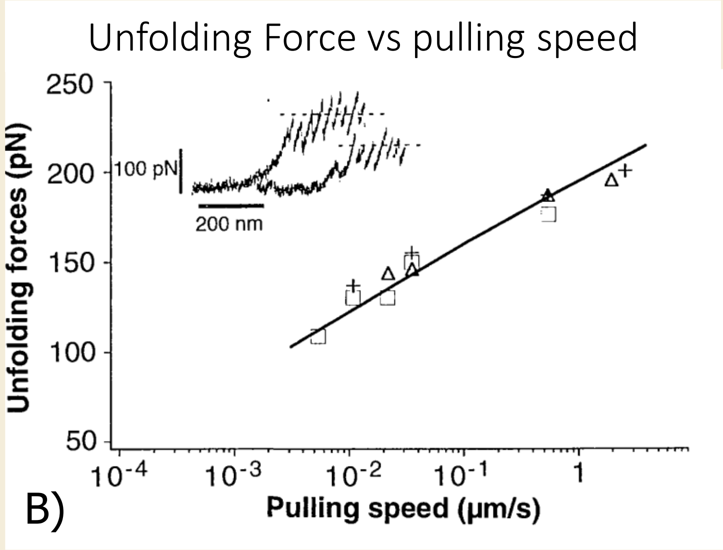
$\delta F_{AFM} >> \delta F_{OT}$

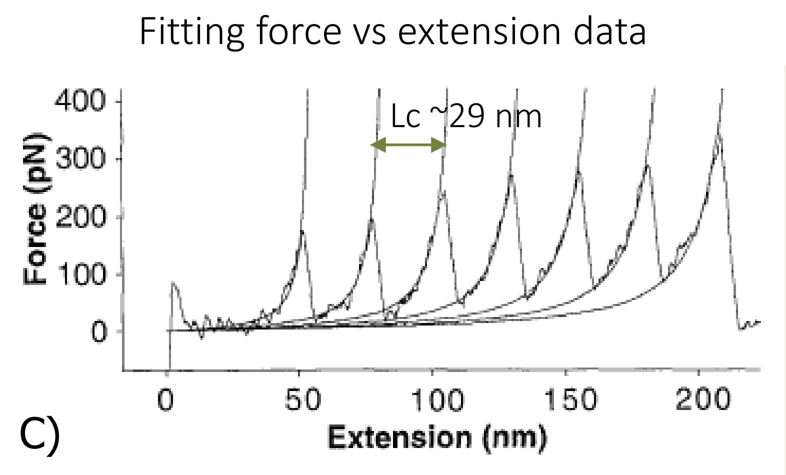
Load rate = probe stiffnes x pulling speed

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

First experiments – unfolding individual immunoglobulin domains of TITIN AFM


Force vs extension curves


Unfolding Force vs pulling speed


Fitting force vs extension data

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 pN/nm, the range of the corresponding load rate is:

r= 0.1 – 10 nN/s .

Ex: V= 1000 nm/s → r= 10 nN/s

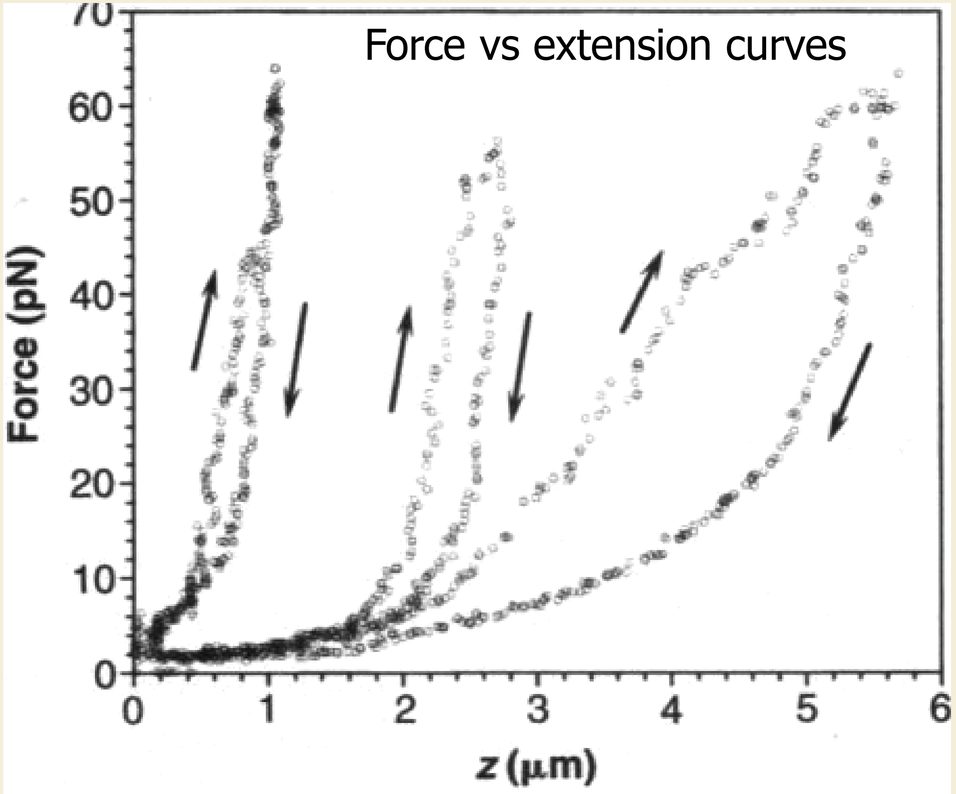
C) Fitting with the WLC (Worm Like Chain) model for each Ig domain unfolding

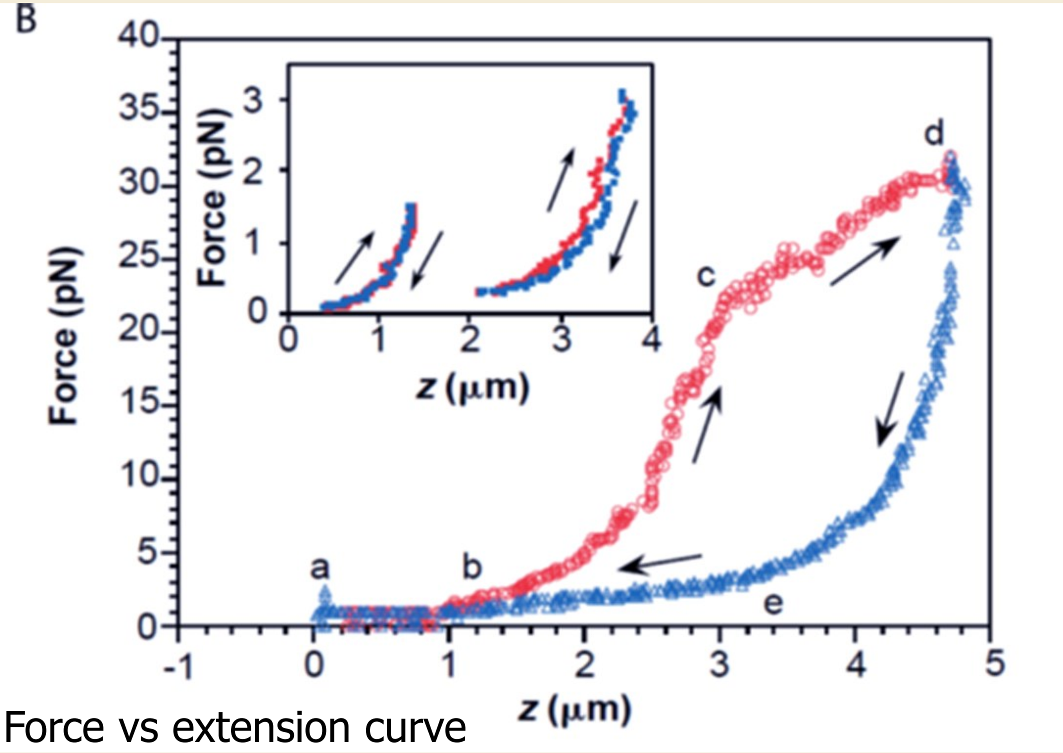
$$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{2} - \frac{1}{4} + \frac{x}{L_c}\right]$$

Persistence length p ~ 0.4 nm
Contour length Lc ~ 29 nm

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

First experiments – unfolding single molecule TITIN **OT**





Force vs extension curve

A) Stretch and release of single titin molecules at a load rate r < 0.05 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 transistions are highlighted. At the beginning of the stretch 5-4% of the 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;

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).

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 <u>distance to the transition state</u>, the <u>height of the energy barrier</u>, and the <u>energy difference</u> 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.

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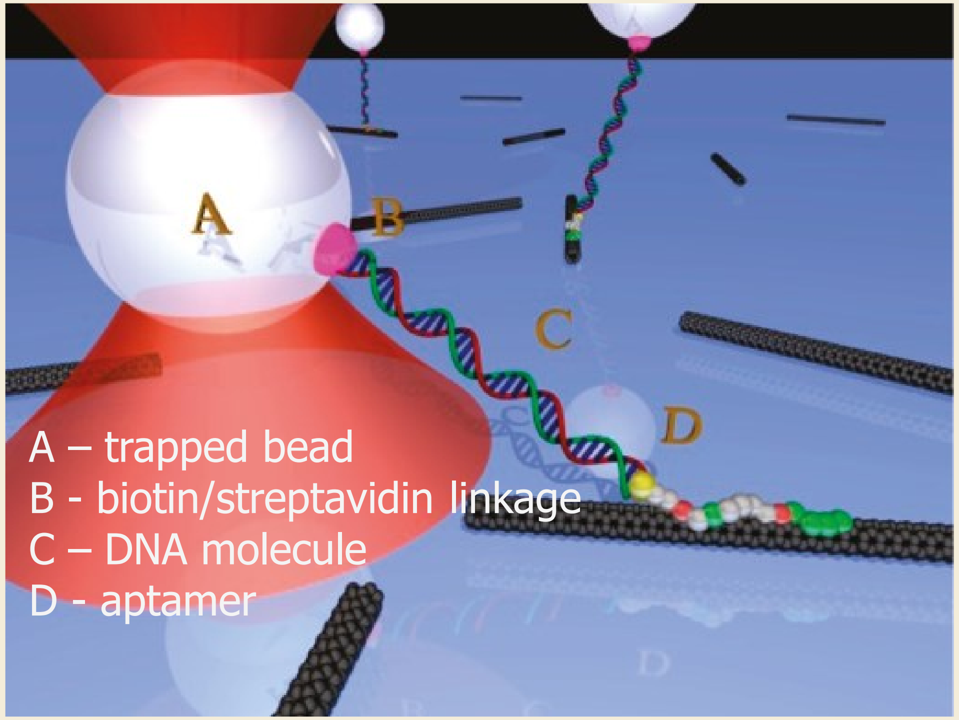
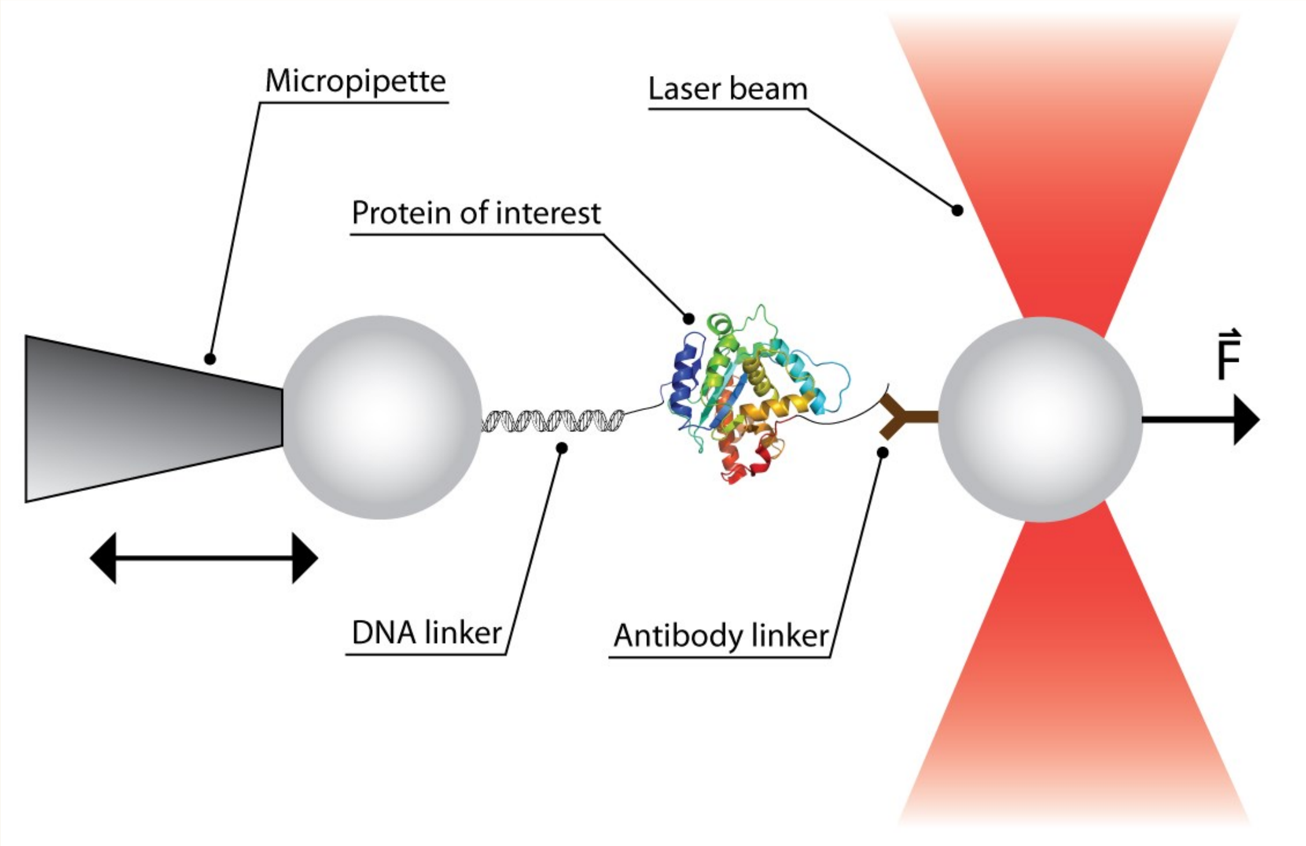
Carlos Bustamante et all Review

Examples of OT configuration for force spectroscopy



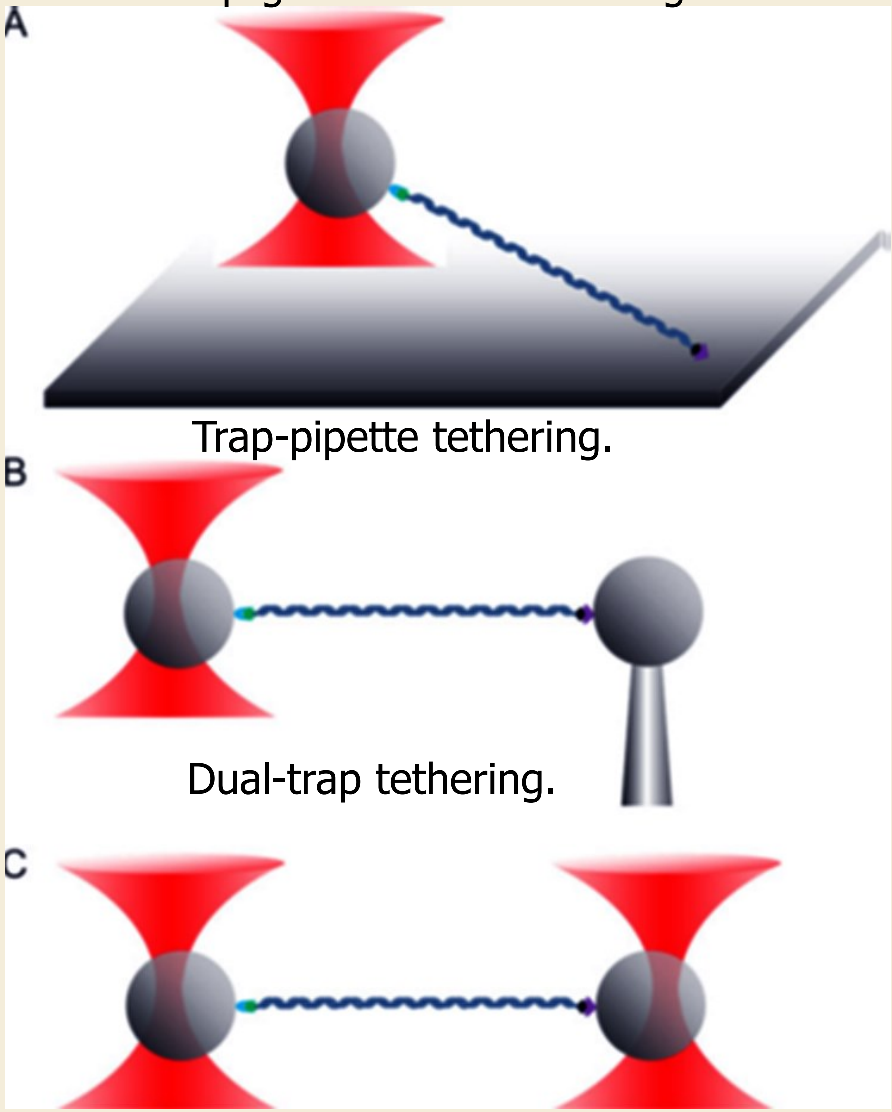
A – trapped bead
B - biotin/streptavidin linkage
C – DNA molecule
D - aptamer



Substrate → Bead- micropipette (far from substrate).

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

OT configurations for force spectroscopy



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 and it is possible)

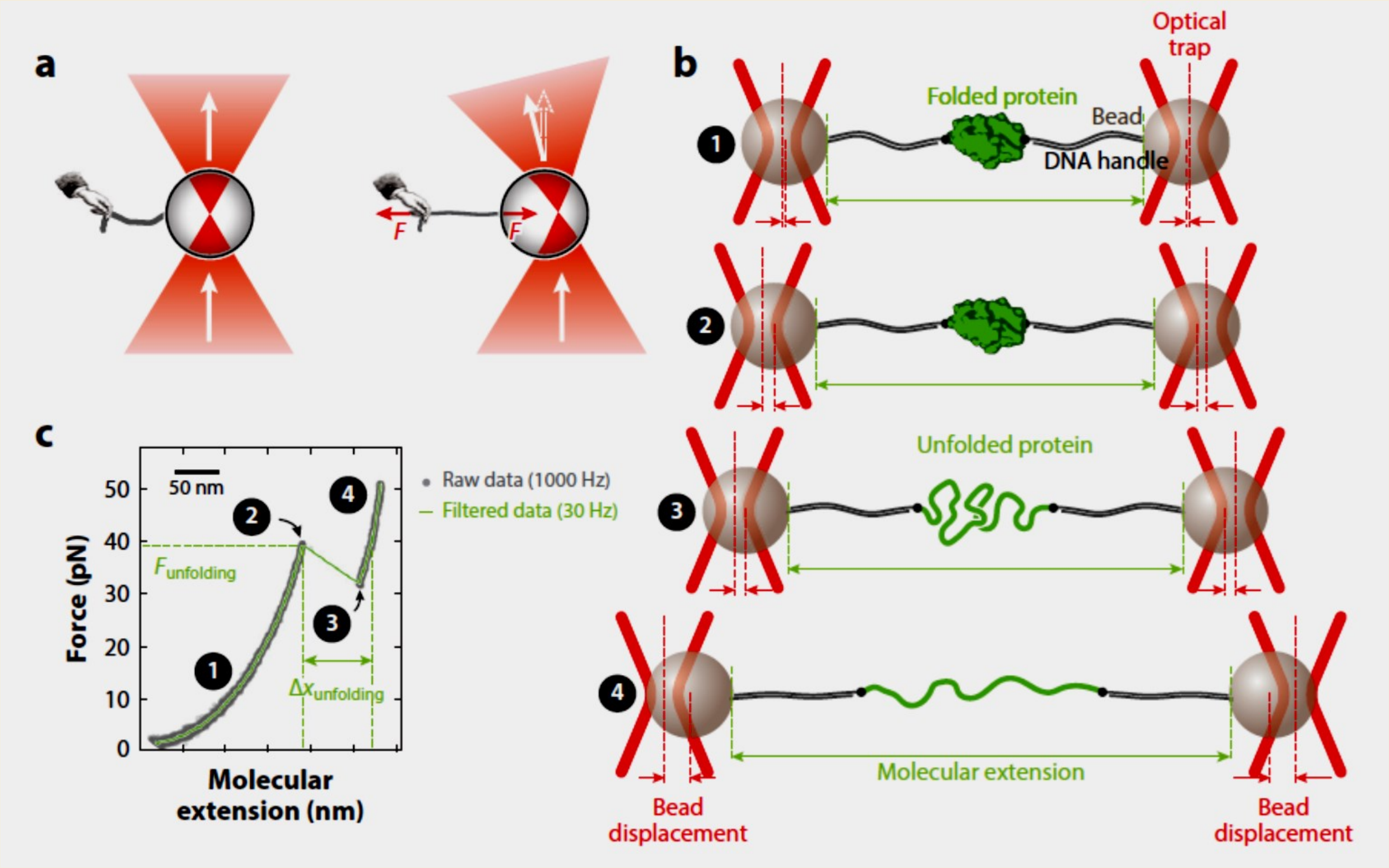
Each of these geometries can be implemented as:

- Force ramp F(t)= r*t (r= ct)
- Force clamp F(t)= ct
- Position clamp x(t)= ct

$F = k\,x$

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

Manipulation of single molecules using dual OT.



2020 Annual Review of Biochemistry
Single-Molecule Studies of Protein Folding with Optical Tweezers
Carlos Bustamante et all Review

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).

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Single-Molecule Studies of Protein Folding with Optical Tweezers
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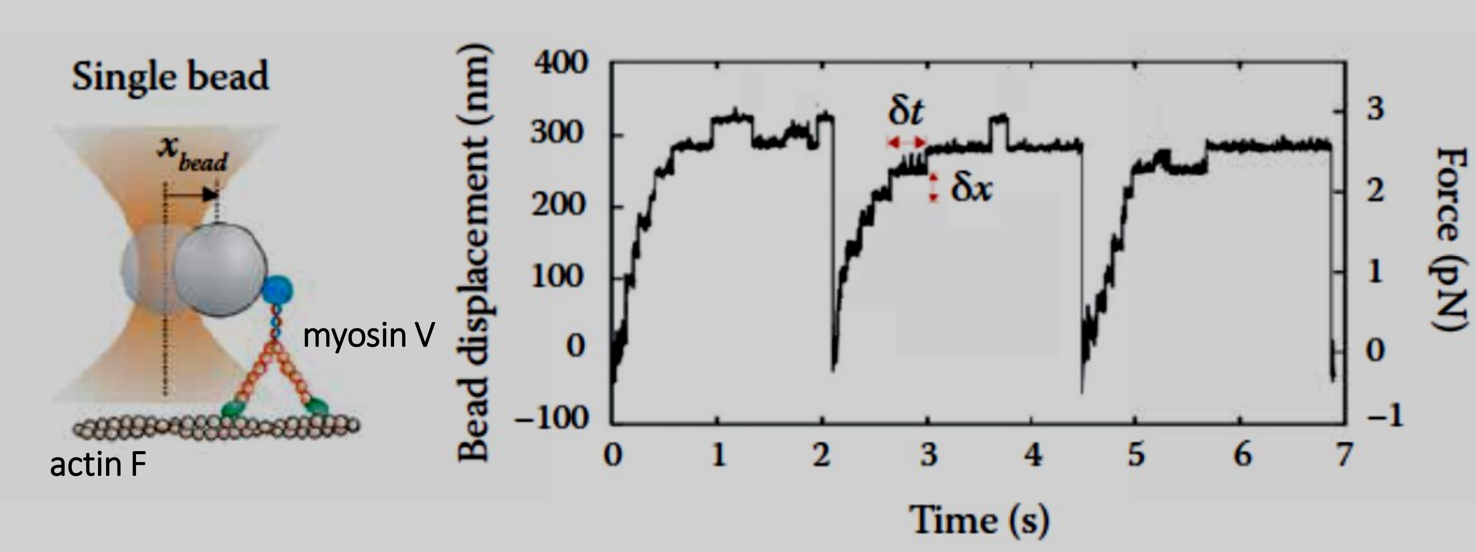
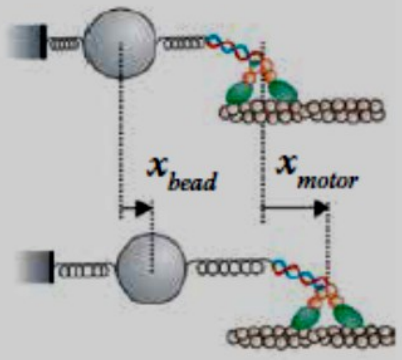
Single-trap geometry. Example.



Uemura, S., et al., Nat. Struct. Mol. Biol., 11, 877–883, 2004



$$x_{bead} = x_{motor} \frac{k_{motor}}{k_{motor} + k_{trap}}$$

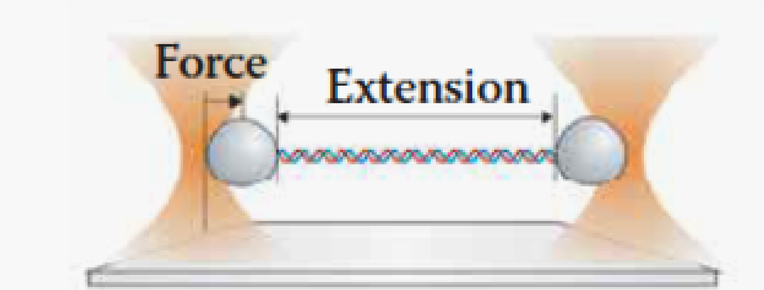
A myosin V molecule is attached to a trapped bead which proceeds along an actin filament stuck on the coverslip surface.
The optical trap position is fixed and the bead displacement x_{bead} measures protein displacement.

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.

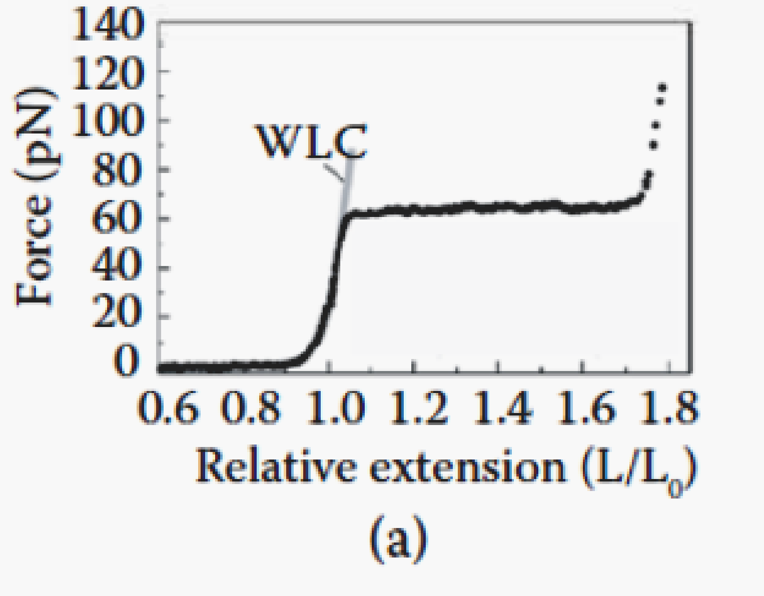
Measured bead displacements (x_{bead}) do not correspond to motor displacements (x_{motor}) but depend on the motor protein displacement and on the values of the trap and protein stiffness.

Capitanio, M . Book Chapter 2017

More flexible and precise
(SNR, temporal and spatial resolution)



Force–extension curve of a biopolymer.



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%.

van Mameren, J., et al., Proc. Natl. Acad. Sci. U S A, 106, 18231–18236, 2009

RNA polymerases and DNA transcription

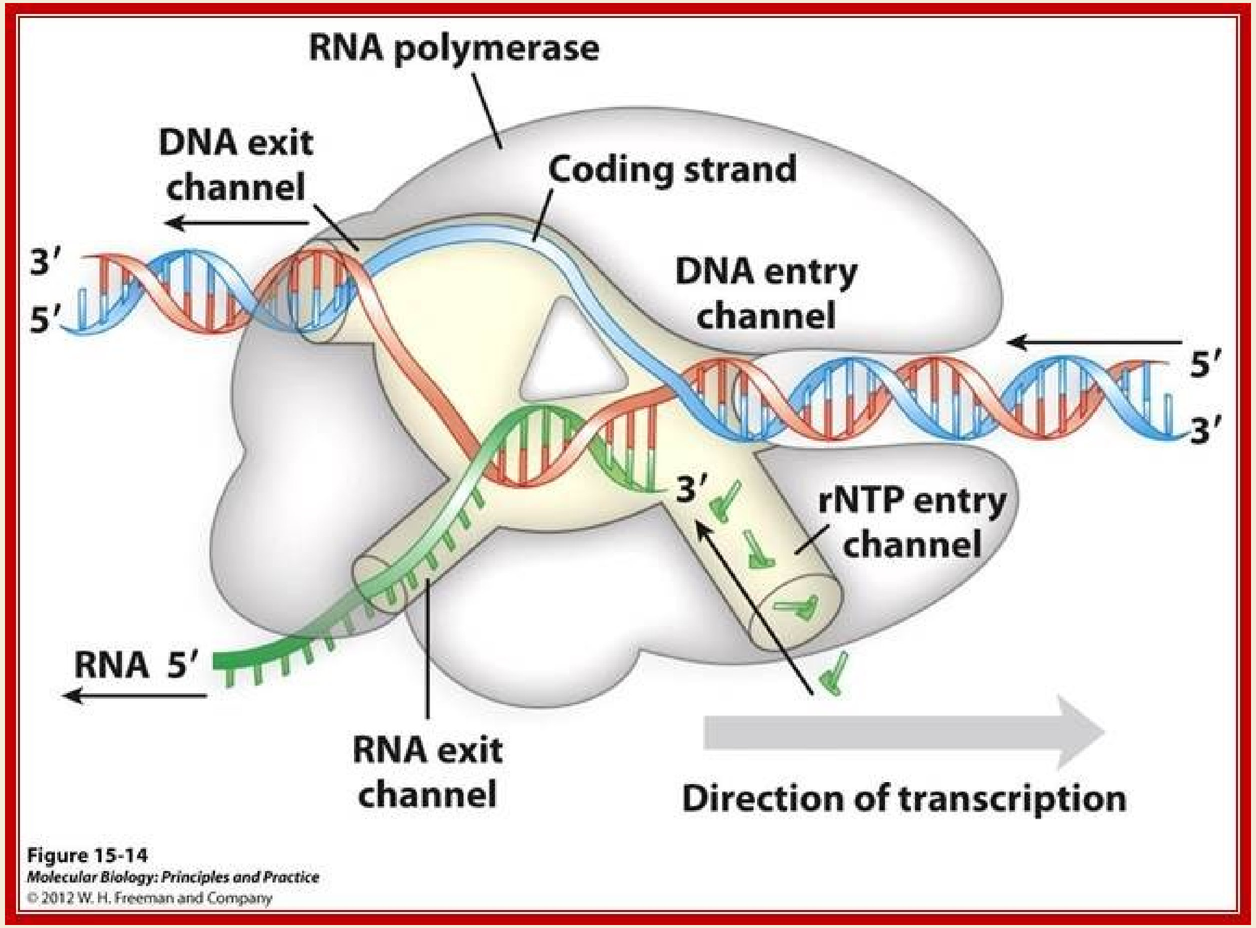


Figure 15-14
Molecular Biology: Principles and Practice
© 2012 W. H. Freeman and Company

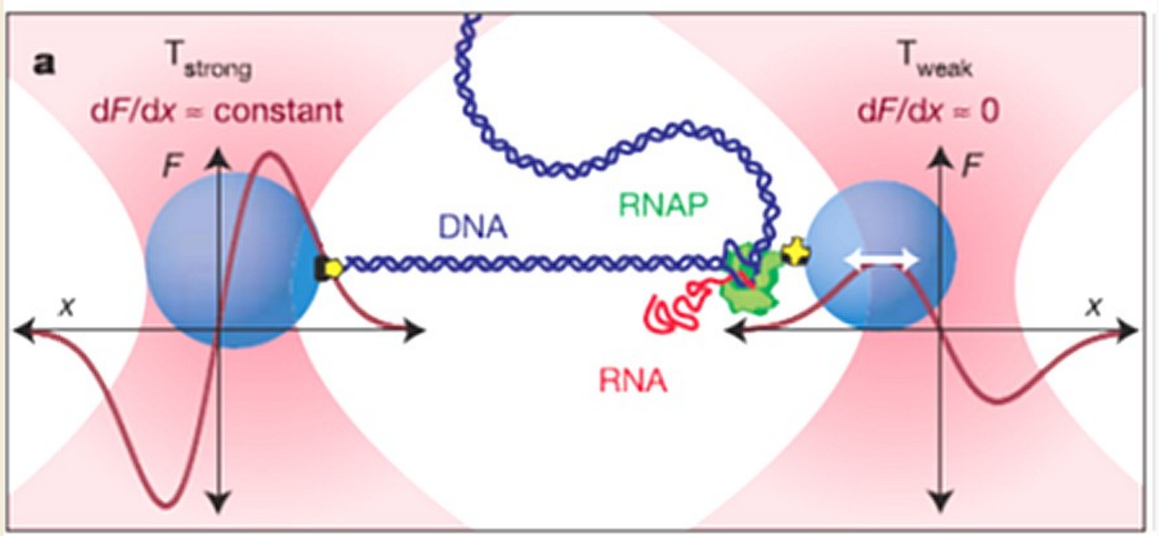
RNA polymerase is the main transcription enzyme.
It transcribes DNA into RNA

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.

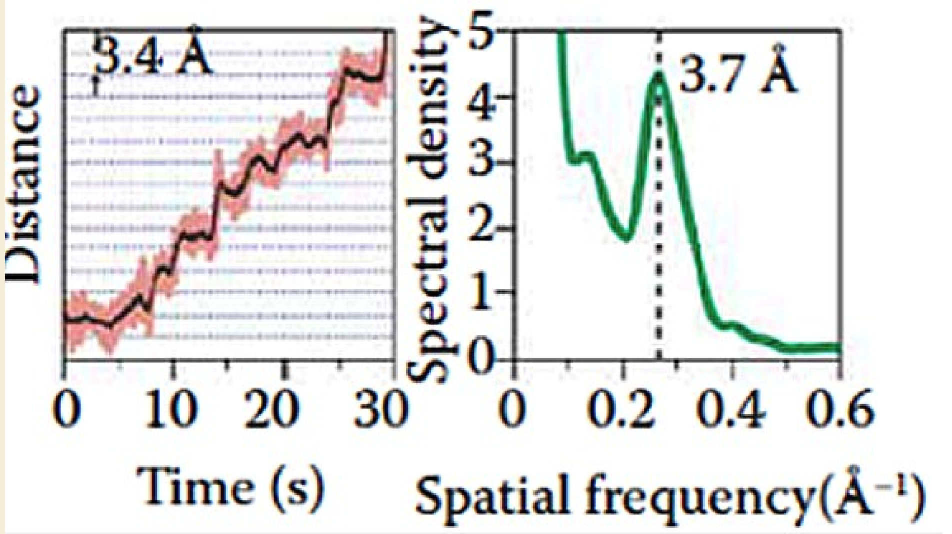
RNAP needs to have the substrate nucleoside triphosphate (NTP) diffuse into its deeply buried active site.

Double-trap assay.



Stiff trap

Weak trap



Example: Dynamics of DNA-processing enzymes.

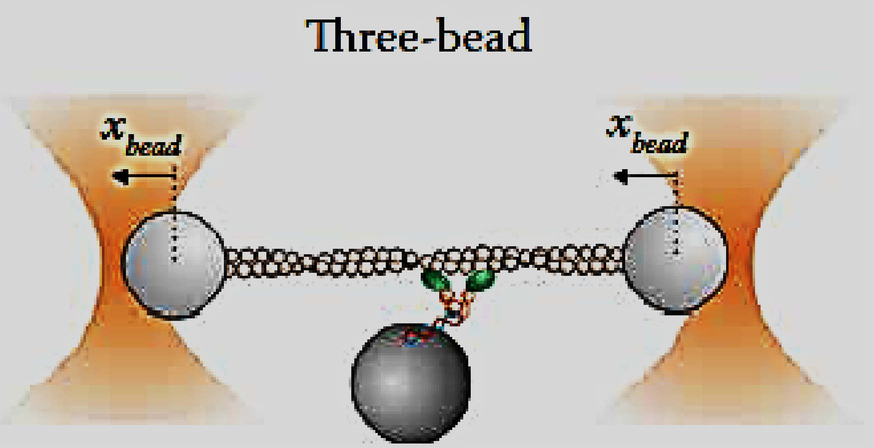
Single, transcriptionally active molecule of RNA polymerase (RNAP, green) attached to a bead held in a trap and tethered via the upstream DNA to another trapped bead. During elongation, the DNA tether lengthens and the beads move apart.

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)

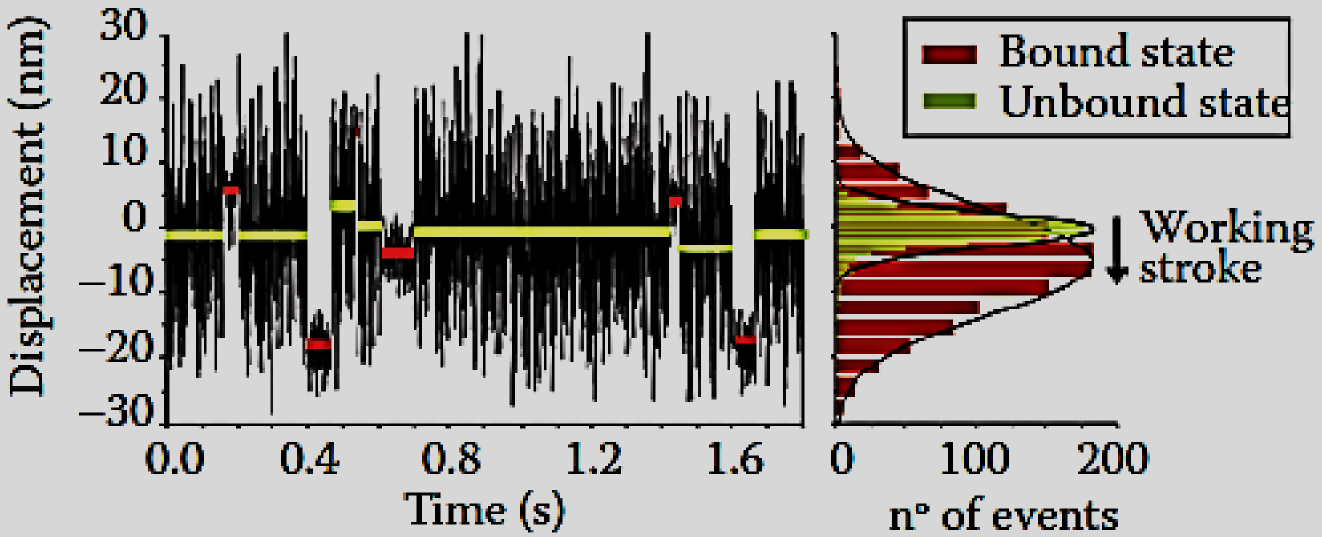
The power spectrum of the average autocorrelation function derived from position histograms shows a peak at the spatial frequency corresponding to the inverse of the fundamental step size, 3.7 ± 0.6 Å.

Abbondanzieri, E.A., et al., Nature, 438, 460–465, 2005

Three-bead assay. Example: working stroke of myosin



Actin filament connected to polystyrene beads held by optical tweezers.



Position recording while myosin is interacting with actin.

A single skeletal myosin II 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.

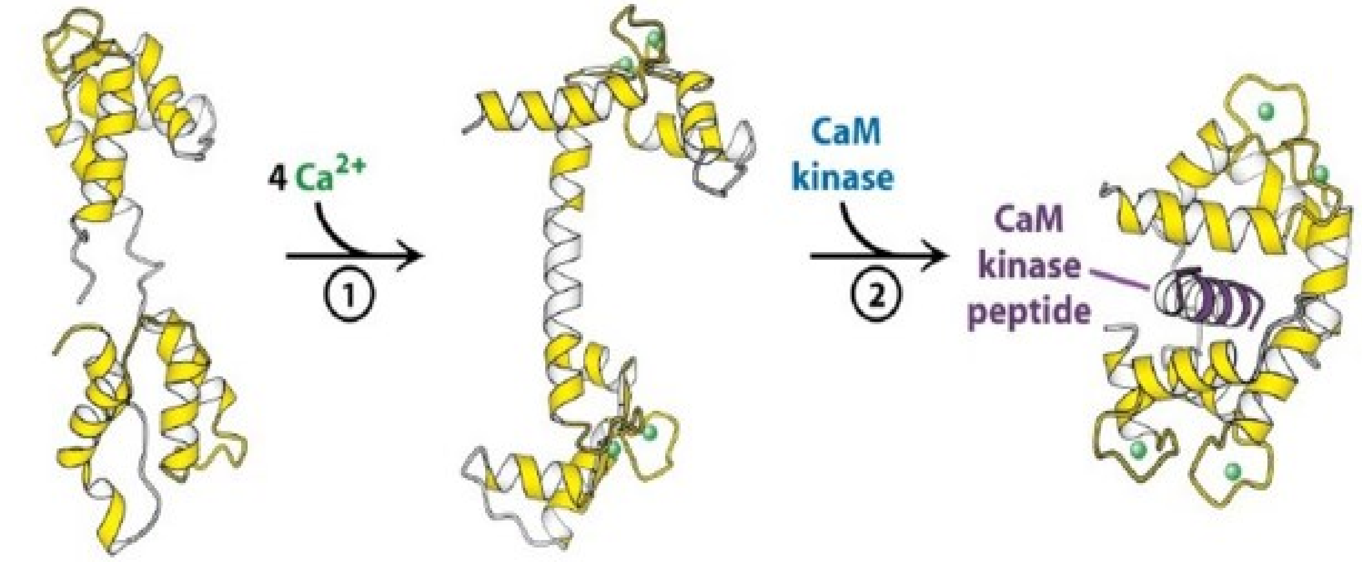
Red and yellow lines indicate the average position of bound and unbound events, respectively.

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

Capitanio, M., et al., Micr. Res. Tech., 65, 194–204, 2004

Example of application : Calmodulin folding-unfolding energy landscape



Calmodulin (apo)

Figure 14-16b
Biochemistry, Sixth Edition
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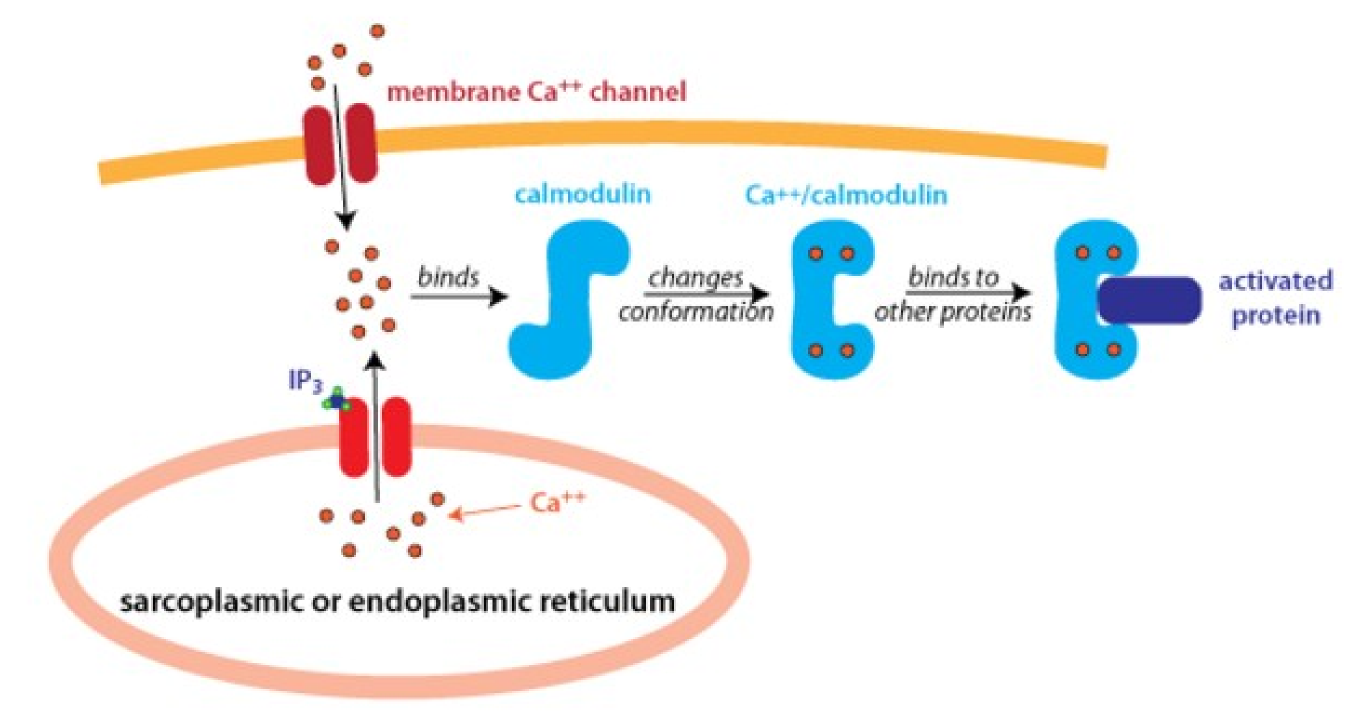
Calmodulin CaM is a protein with a molecular mass of 16 kDa, consisting of 148 amino acid residues and is characterized by a helix-loop-helix binding motif, also known as the E-F hand motif.

CaM has one subunit with a distinct dumbbell shape in which a linker region joins two globular domains.

- CaM is known to undergo a conformational change upon binding with a calcium ion in which each lobe transitions from a closed conformation to an open conformation.
- CaM has four major, high-affinity binding sites.
- The CaM binding region is a series of hydrophobic amino acids (such as Trp or Leu), hydrophilic amino acids (such as Glu or Asp), and basic amino acids (such as Arg or Lys). 12 - Ionic bonds – about 100 KT strength
- CaM typically wraps around its target, with the two globular domains gripping either side of it.

Calmodulin Pathway simplified



Ca++ cell exterior – interior influx, or release from the Endoplasmatic Reticulum

Calmodulin binds to 4 Calcium Ions and undergoes conformational changes characterized by different states in the free energy landscape

→ activates other proteins

Calmodulin folding-unfolding energy landscape

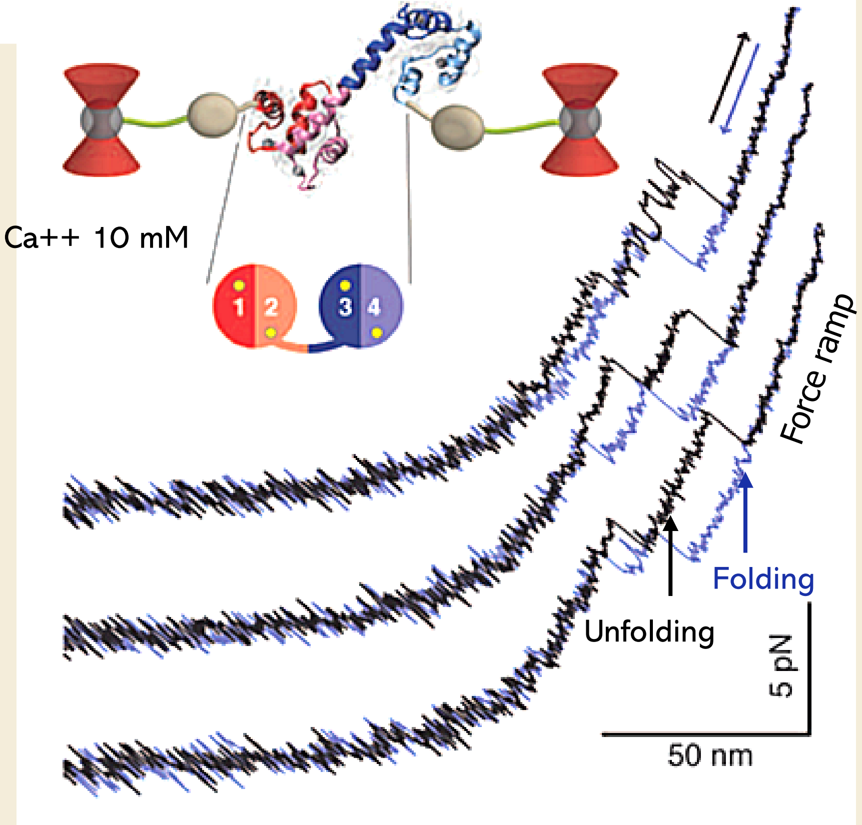
Ref: The complex folding network of single calmodulin molecules, Stigler, J., et al., Science, 334, 512–516, 2011

The work shows that between the unfold and folded states there are also other (intermediate) states

Calmodulin folding-unfolding energy landscape – Optical Tweezers

Sketch of the experimental setup with
the protein linked with ubiquitin-DNA handles

Silica bead bead streptavidin – DNA biotin -
Ubiquitin protein + cysteine residue - CaM domain



+ Ca++ 10 mM

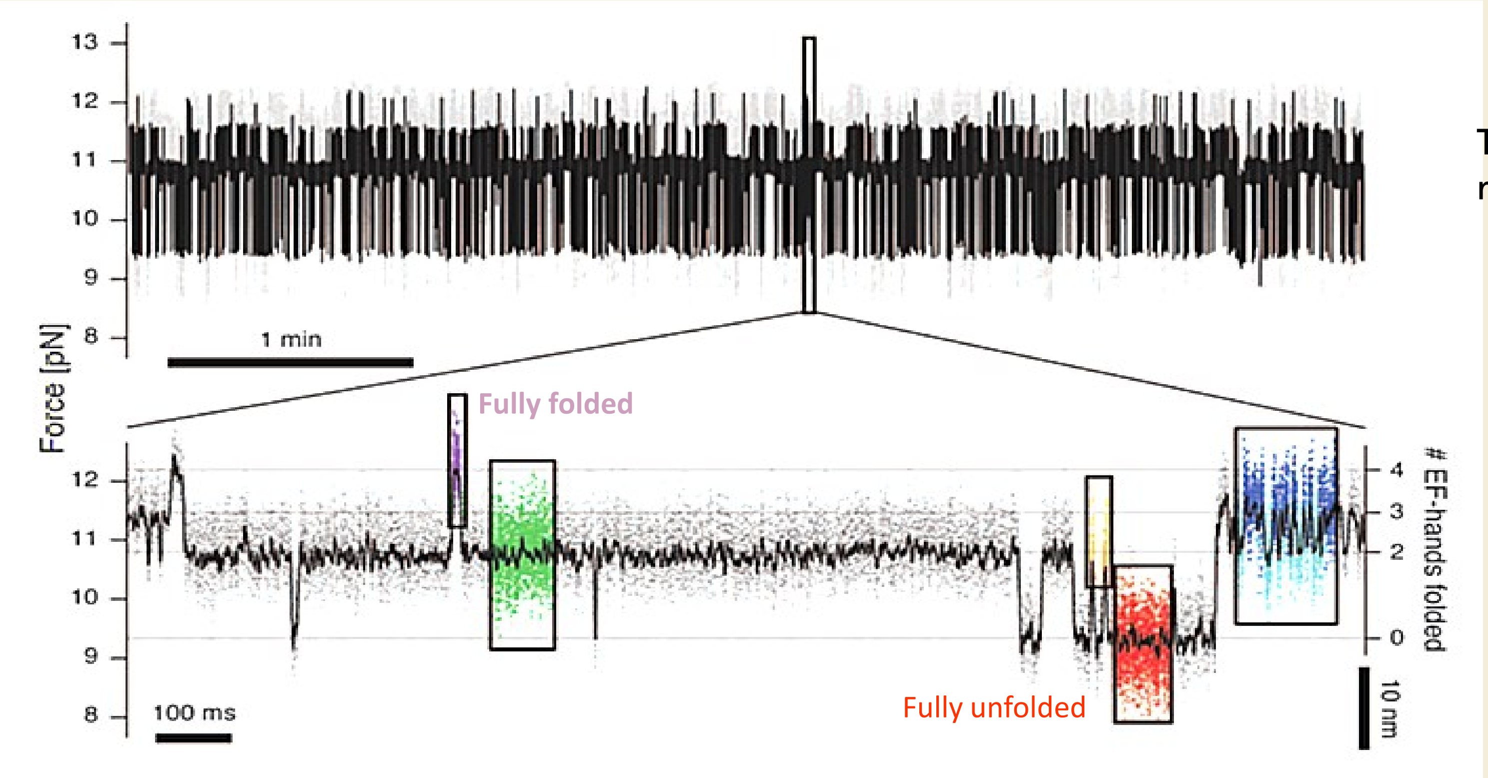
Dual OT
k= 0.25 pN/nm; v= 500 nm/s; r = 12.5 pN/s
Data collection 100 kHz, averaged to 20 kHz before storage

Representative stretch-relax cycles for CaM showing unfolding - folding of the two globular domains of the CaM

The rapid oscillations in the upper traces provide indications for deviations from a simple two-step unfolding behavior

The complex folding network of single calmodulin molecules, Stigler, J., et al., Science, 334, 512–516, 2011

Sample trace during 5min of the fluctuations of a singleWT-CaM molecule at a pretension force set at 11 pN



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

Six different states can be identified

See suplem info for details on states classification using Markov analysis

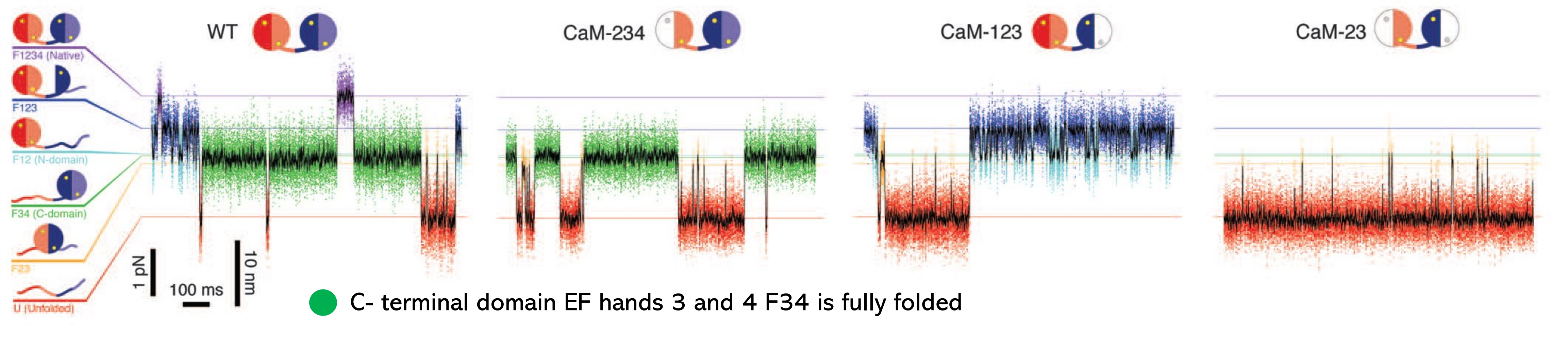
● ● ● Length expected for a state with 2 EF hands folded and 2 EF hands unfolded. However, they have different kinetics.

● The extension corresponds to 3 folded EF hands

● Unfolded ● Folded

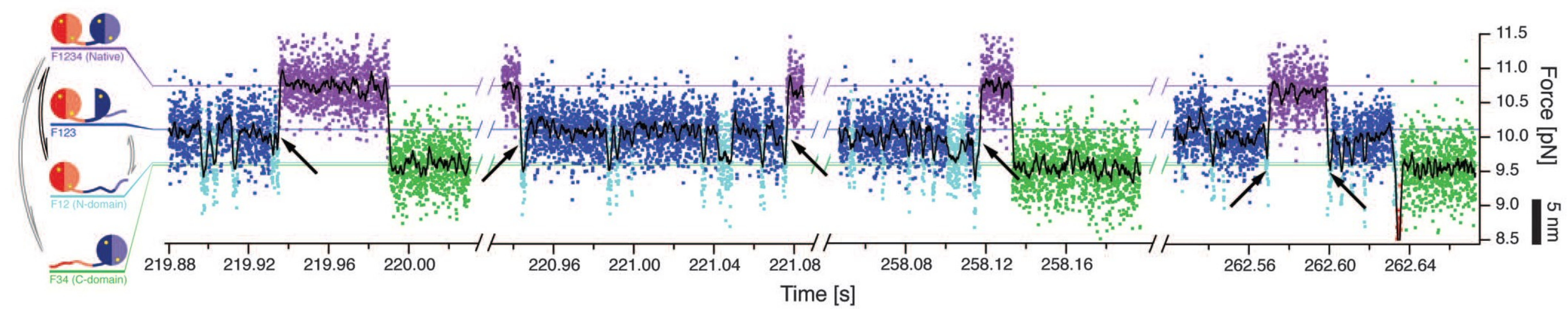
Molecular deletion constructs were used to decipher the structures underlying the various states

State assignment and comparison of WT traces with truncation mutants



● C- terminal domain EF hands 3 and 4 F34 is fully folded

Transitions to and from the native state F1234



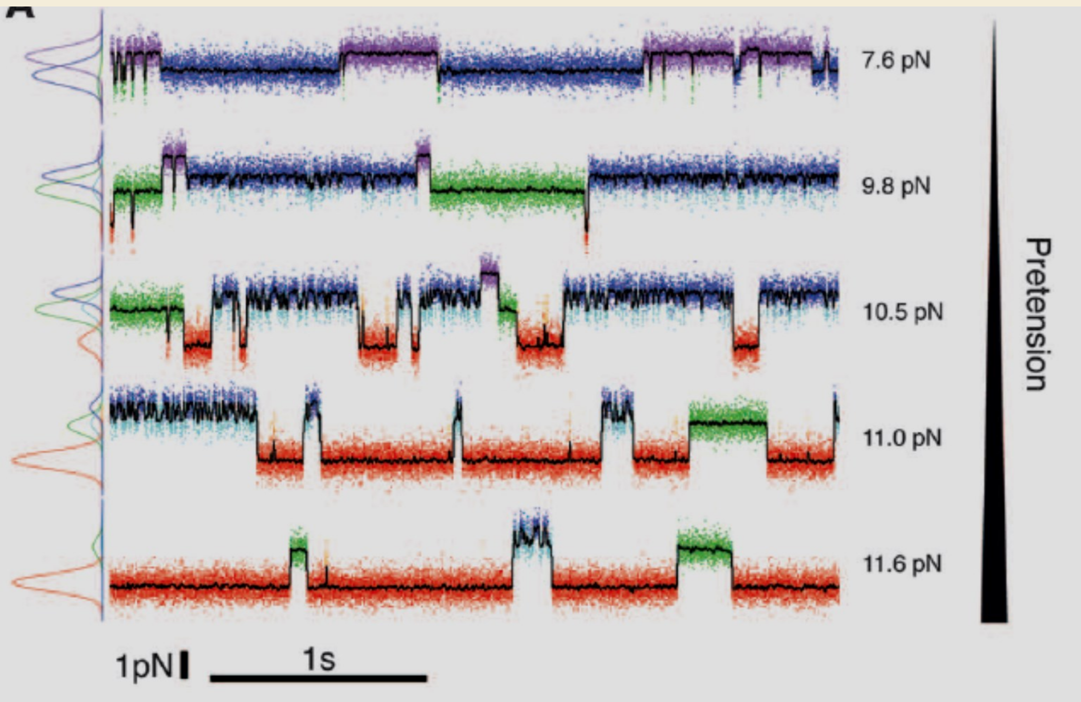
Direct transitions to and from the native state (purple) can only occur to and from states F34 (green) or F12 (light blue).

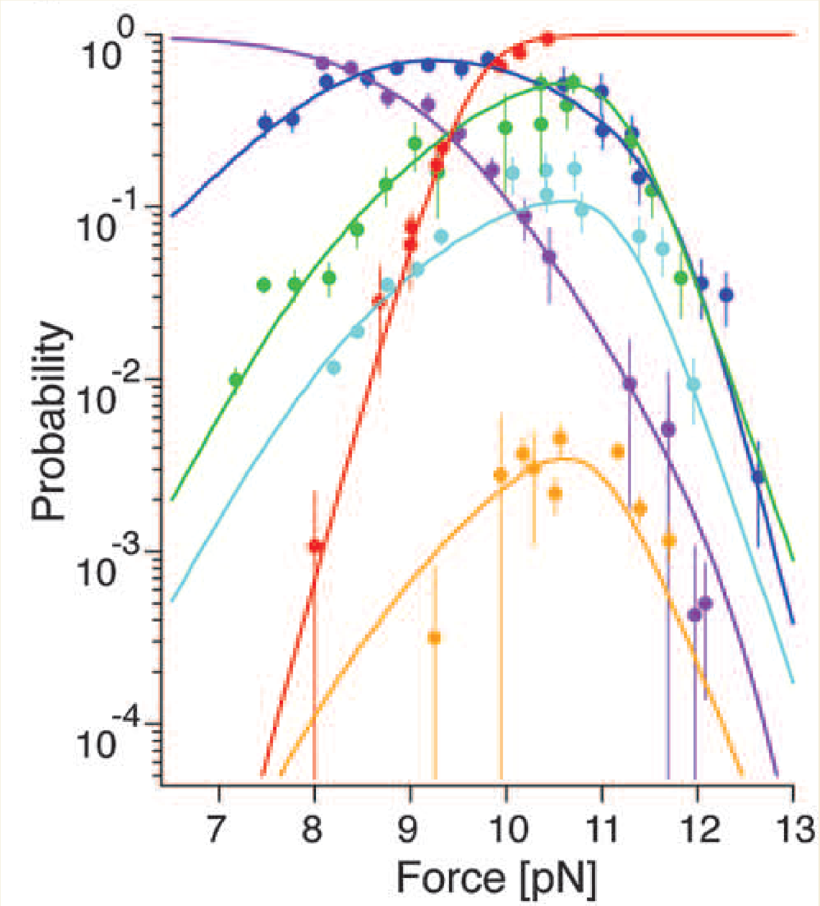
Transitions of F123 (dark blue) to and from F1234 always occur through F12 (see arrows), identifying F123 as an off pathway intermediate.

Observing the transitions between the six states at constant tension / force

Traces of WT-CaM at different pretensions

Probabilities of the states vs Force / Tension

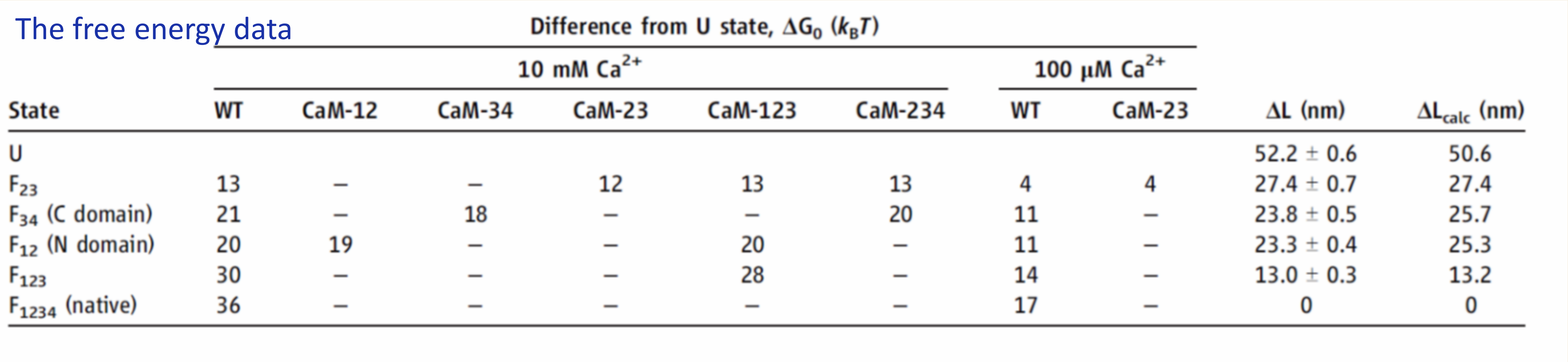




At low pretensions of 7.6 pN folded or largely folded states dominated. The more tension applied, the more unfolded states were populated, until, at 11.6 pN, the unfolded state prevailed.

Observing the transitions between the six states at constant tension / force

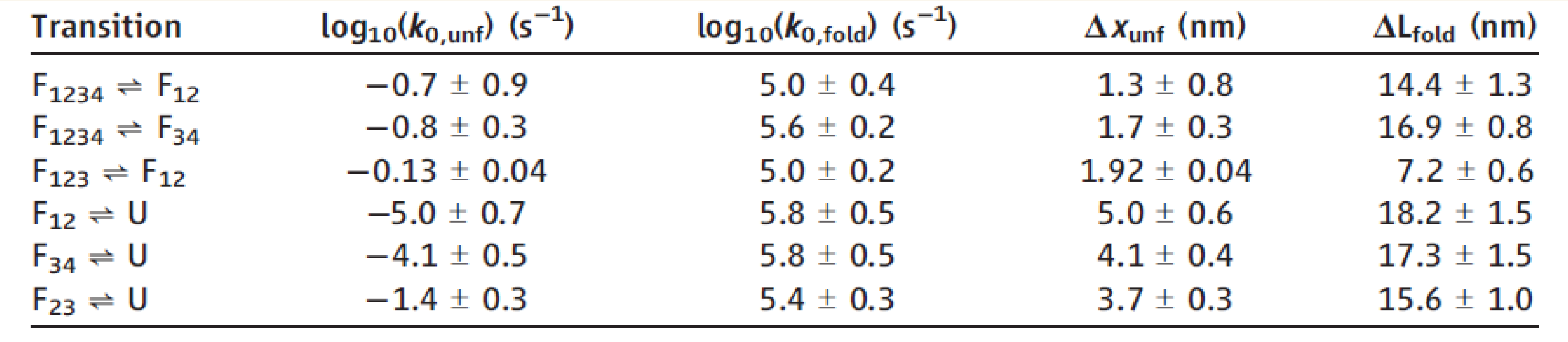
The free energy data



	Difference from U state, ΔG_0 (k_BT)									
	10 mM Ca^{2+}						100 µM Ca^{2+}			
State	WT	CaM-12	CaM-34	CaM-23	CaM-123	CaM-234	WT	CaM-23	ΔL (nm)	ΔL_{calc} (nm)
U									52.2 ± 0.6	50.6
F_{23}	13	–	–	12	13	13	4	4	27.4 ± 0.7	27.4
F_{34} (C domain)	21	–	18	–	–	20	11	–	23.8 ± 0.5	25.7
F_{12} (N domain)	20	19	–	–	20	–	11	–	23.3 ± 0.4	25.3
F_{123}	30	–	–	–	28	–	14	–	13.0 ± 0.3	13.2
F_{1234} (native)	36	–	–	–	–	–	17	–	0	0

The data reveals a significant anti cooperativity between the folding of the two CaM domains.
From the unfolding state, folding of either N terminal (F12) or C terminal (F34) domain results in an energetic gain 20 KT.
However, folding of a second domain to the native state provides 15 KT.
Apparently, the presence of one folded domain prevents the other domain from reaching its energetically optimal state.

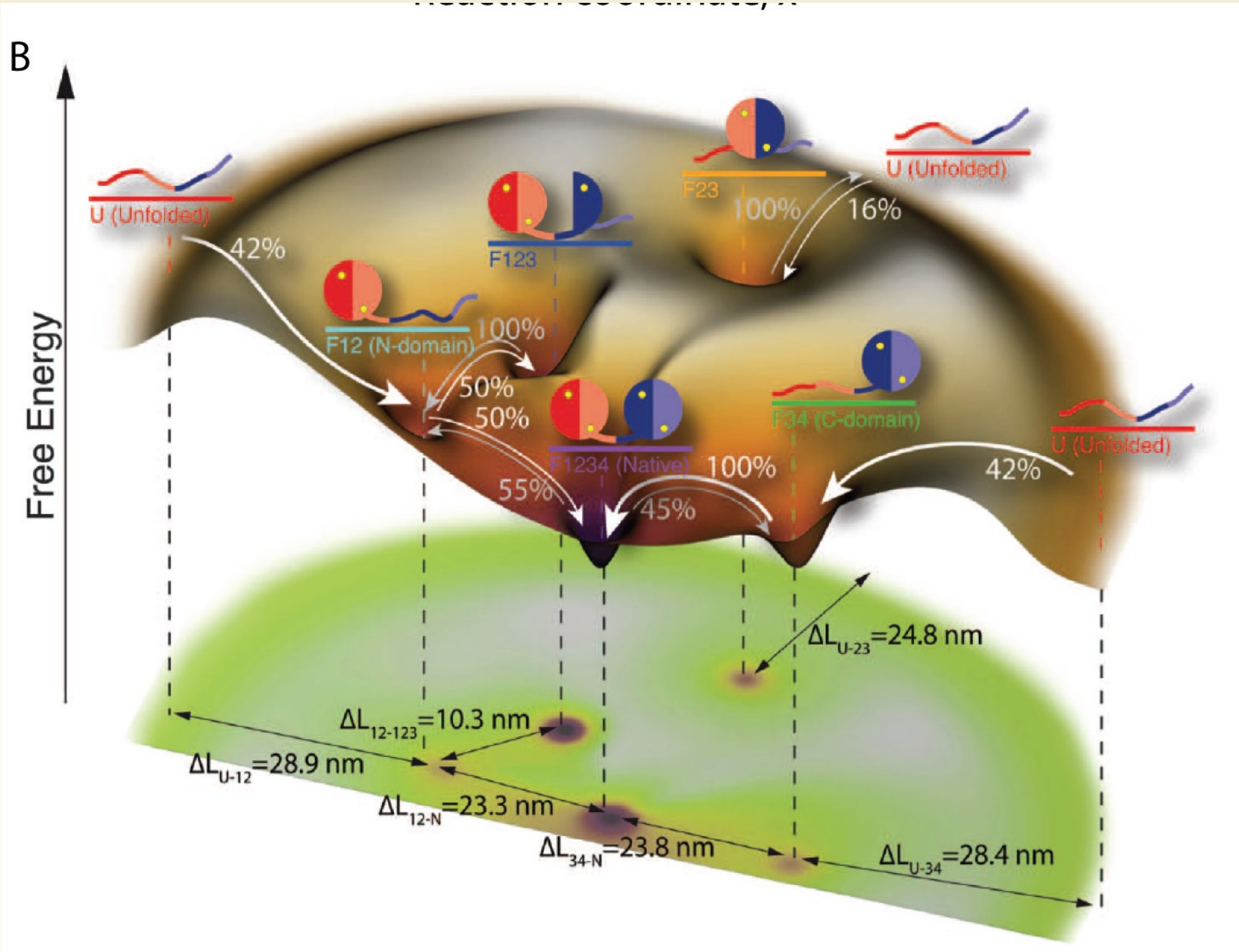
Transition constants



Transition	$\log_{10}(k_{0,unf})$ (s^{-1})	$\log_{10}(k_{0,fold})$ (s^{-1})	Δx_{unf} (nm)	ΔL_{fold} (nm)
$F_{1234} \rightleftharpoons F_{12}$	−0.7 ± 0.9	5.0 ± 0.4	1.3 ± 0.8	14.4 ± 1.3
$F_{1234} \rightleftharpoons F_{34}$	−0.8 ± 0.3	5.6 ± 0.2	1.7 ± 0.3	16.9 ± 0.8
$F_{123} \rightleftharpoons F_{12}$	−0.13 ± 0.04	5.0 ± 0.2	1.92 ± 0.04	7.2 ± 0.6
$F_{12} \rightleftharpoons U$	−5.0 ± 0.7	5.8 ± 0.5	5.0 ± 0.6	18.2 ± 1.5
$F_{34} \rightleftharpoons U$	−4.1 ± 0.5	5.8 ± 0.5	4.1 ± 0.4	17.3 ± 1.5
$F_{23} \rightleftharpoons U$	−1.4 ± 0.3	5.4 ± 0.3	3.7 ± 0.3	15.6 ± 1.0

Dx unf is the change in the length required to reach the transition state of unfolding.
DL fold is the contour length change required to reach the transition state of folding.

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.

The complex folding network of single calmodulin molecules, Stigler, J., et al., Science, 334, 512–516, 2011

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 : $k = 1$ pN /nm → τ – microseconds , OT : $k= 0.001$ pN/nm → τ - milliseconds

CELL MECHANICS

LECTURE 4

SUMMARY

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

Reference: Book_Howard_Ch_5 + others see slides

SUMMARY

Lecture 4 SUMMARY

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

- Chemical forces; amino acids – protein; types of forces;
- Ligands: how they work and examples (oxygen, rhodopsin)
- Chemical equilibria and the effect of force on chemical equilibria (ex hair cells)
- Rate theory of chemical reactions and the effect of force on the chemical rate constant (ex myosin)
- Examples of single molecule force spectroscopy experiments

  - How to determine the dissociation rate of a ligand – receptor bond using force
    (model and exp Adhesion through Single Peptide Aptamers
  - Titin unfolding AFM vs OT
  - OT configurations for force spectroscopy – single molecule experiments examples
  - The complex folding network of single calmodulin molecules using OT single molecule force spectroscopy