

Molecules that are capable of fluorescing are called *fluorescent molecules*, *fluorescent dyes*, or *fluorochromes*. If a fluorochrome is conjugated to a large macromolecule (through a chemical reaction or by simple adsorption), the tagged macromolecule is said to contain a *fluorophore*, the chemical moiety capable of producing fluorescence.

UV light in Tonic water emits blue light

Eye is sensitive to blue and not UV



Blue light in Floursine Dye we get yellow fluorescence



Examples of FLUORESCENCE

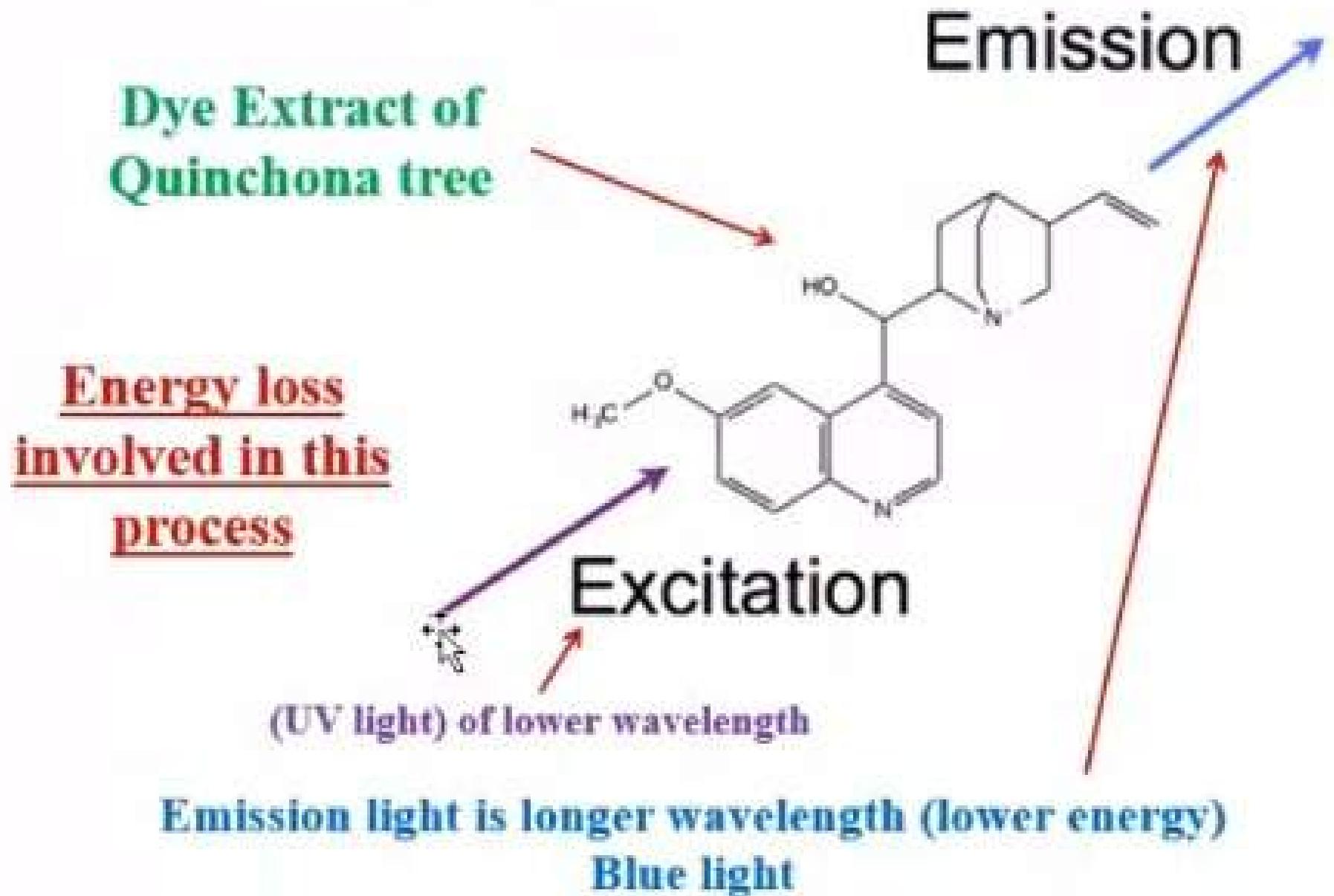
Shining green light in Rodemin dye you get orangey yellowish fluorescence



FLOURESCENCE EXPERIMENT SHOWN

Excitation/Emission

Excitation/Emission



Why Fluorescence?

To get a nice image with parameters below

- High Contrast

- High Specificity

Attach fluorescent labels to specific proteins, specific molecules, specific places in a cell. Target what we want to see

- Quantitative

If system calibrated, higher signal areas means higher protein content

Part of cell labeled in

GREEN(microtubules) and nucleus is in ORANGE due to dye labeling



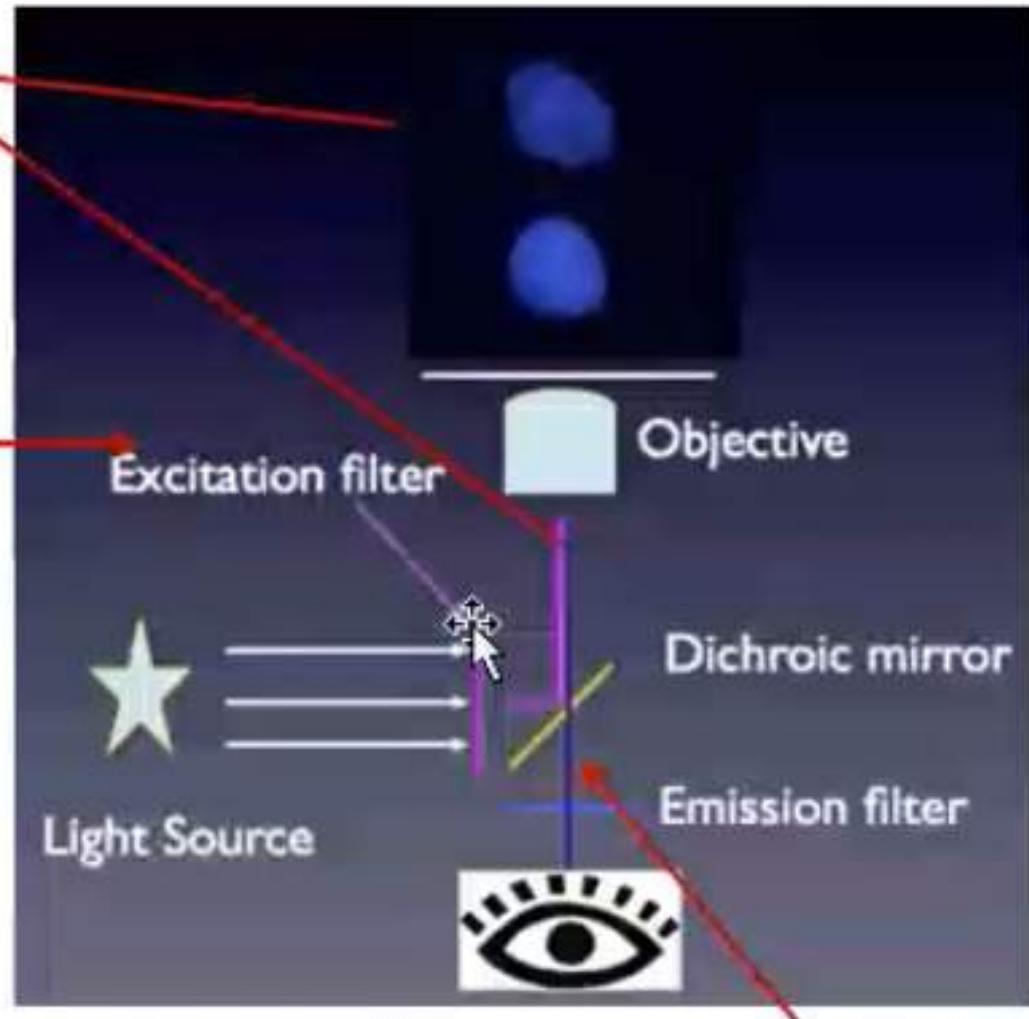
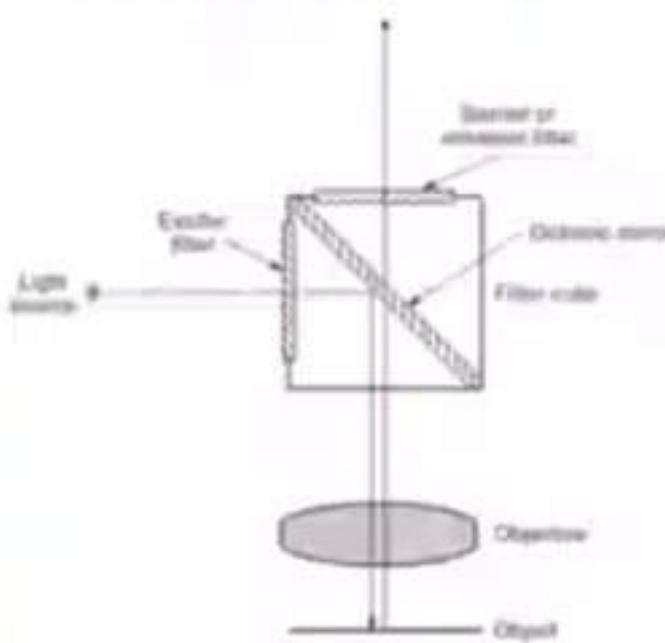
Background is totally black and offers high contrast

Fluorescence Microscope

Filters are very important
and make it different from
a normal microscope

Filters out the source for
wavelength needed

Filters as a cube depicted
from a different source



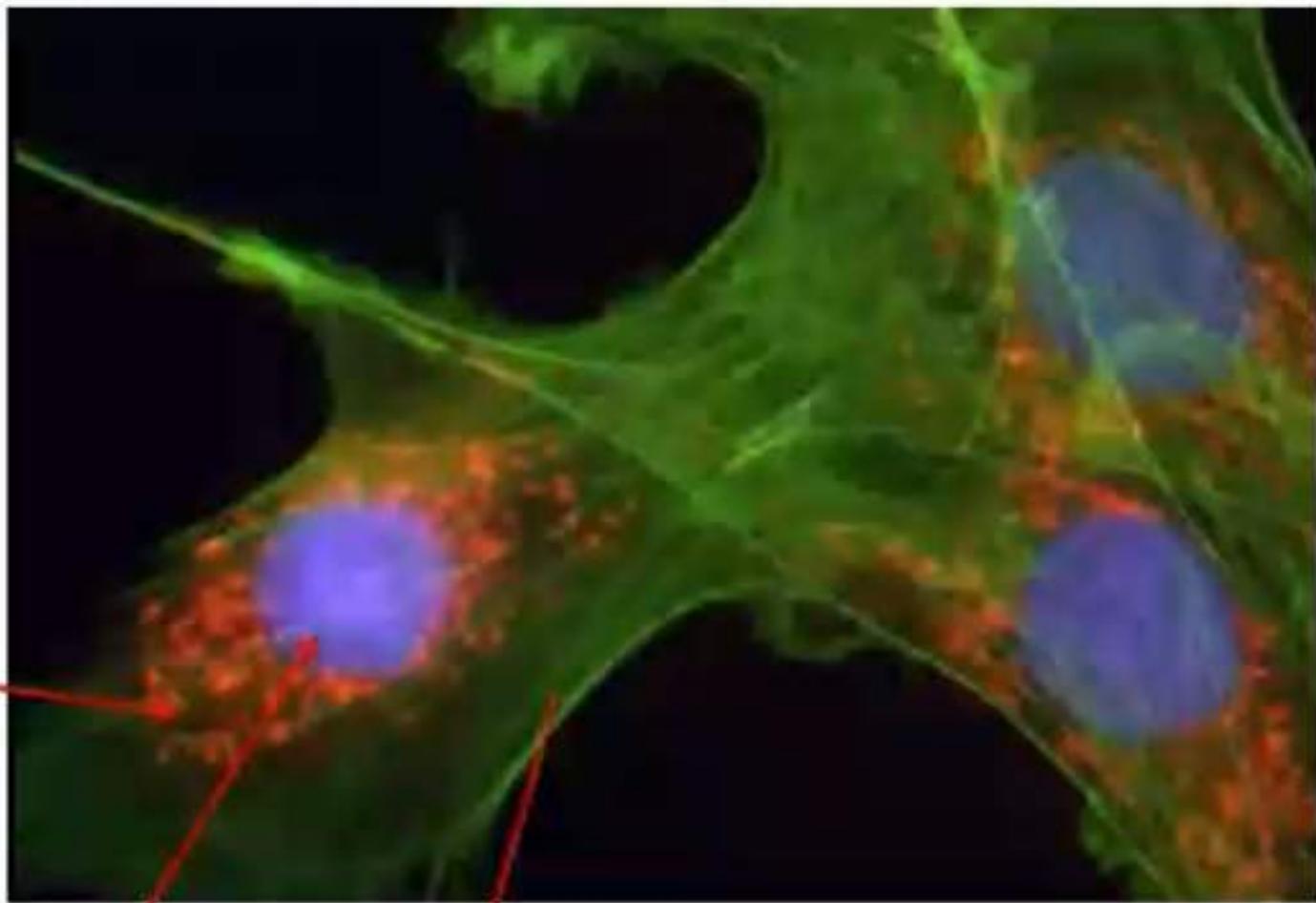
Fluorescence light travels back to eye
through Emission filter

**Images of individuals fluorophores are collected and
Integrate them in a computer**

Mitochondria
staining in RED

DNA in BLUE

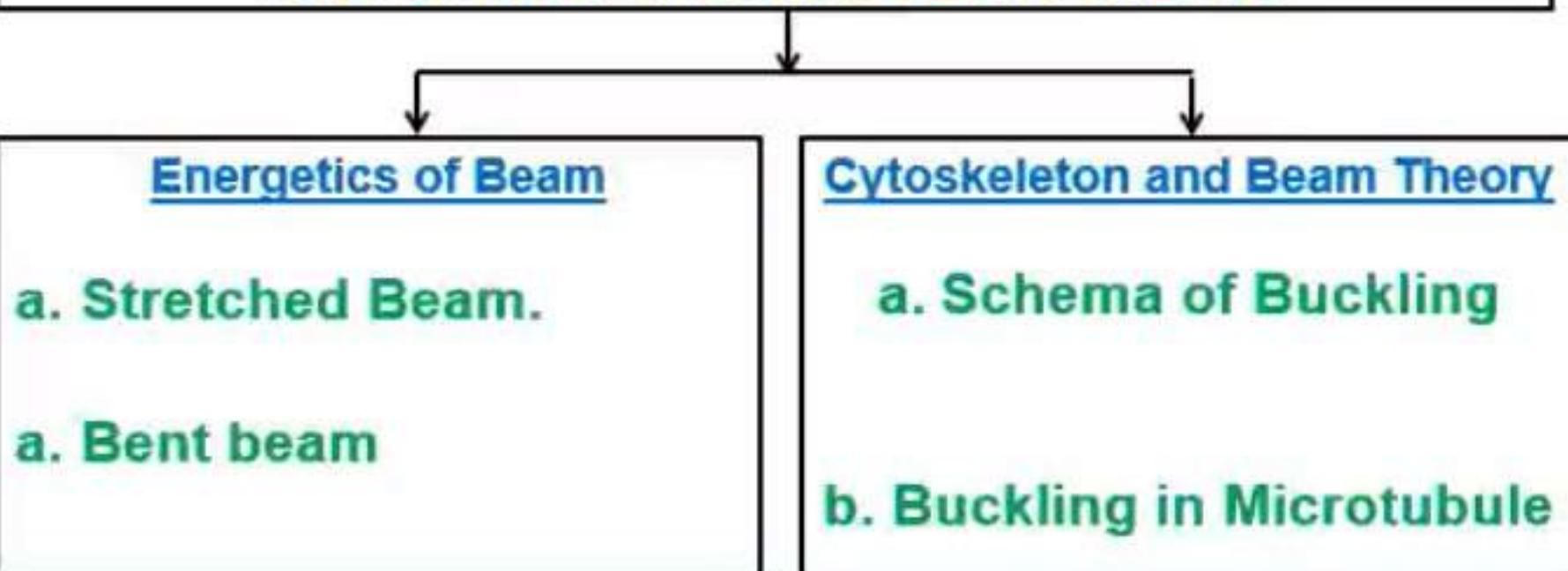
ACTIN stress fibers in
GREEN



**Acknowledgment: This is an Aggregated Lecture from lot of resources
(Textbooks/Coursera/Youtube lectures, Journal Papers)**

TODAYS LECTURE

- a. **QUICK INTRO TO FLUORESCENCE SPECTROSCOPY**
- b. **MODES/ENERGETICS OF BEAM DEFORMATION**
- c. **CYTOSKELETON AND BEAM THEORY**



TODAYS LECTURE



**Do we have Mechanical Structural Elements like
Beams/Cables in our Body**



Beams/Cables are everywhere in our Body (From Flagella to Cytoskeleton)

Many of the macromolecules of living organisms are filamentous. Not only are they a striking visual feature within cells, but their structure is intimately tied to the ways in which such molecules are used in cells. The representation of geometric structures as networks of one-dimensional elements is a perspective of great power and applicability. That part of mechanics which has grown up around this approximation is known traditionally as "beam theory."



Does Mechanical features of Biological structures in our body affect biological function?

Cell Structures viewed as one Dimensional Elastic beams mechanically

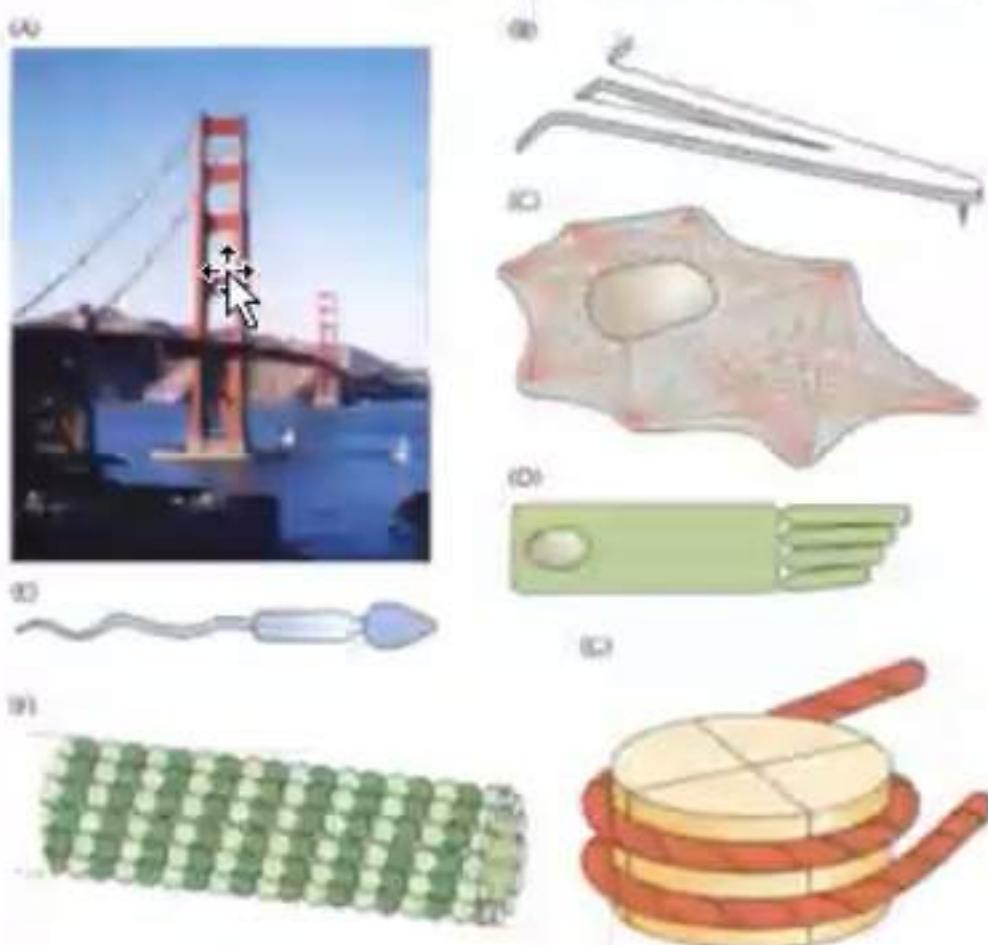


Figure 10.1: Diverse examples of the way in which structures can be interpreted using beam theory.
(A) A bridge as a collection of beams and cables. (B) A small-scale cantilever used in an atomic-force microscope. (C) The cytoskeleton in a eukaryotic cell. (D) Stereocilia on an inner ear hair cell. (E) The flagellum of a sperm. (F) An individual microtubule. (G) A representation of DNA as an elastic rod in the context of the nucleosome.

- Hence we study quantitative framework about structure/energetics for one dimensional beams

Figure from Rob Phillips: Physical Cell Biology



Geometry of Deformation in Biological Structures (Look at with reference to Beam)

Beam Deformation Modes

Biological filaments are characterized by one dimension (the length) that is much greater than their transverse dimensions. For example, in the case of the bacterial flagellum, the structure has a length in excess of microns with a diameter that is measured in only tens of nanometers.

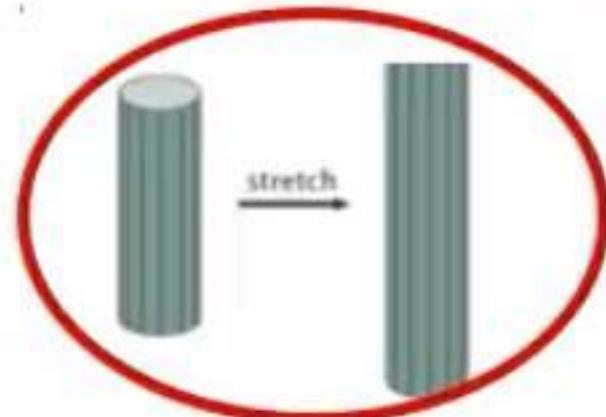


Figure 10.2: Three modes of deformation of a beam: (A) stretching of a beam, (B) bending of a beam, and (C) twisting of a beam.

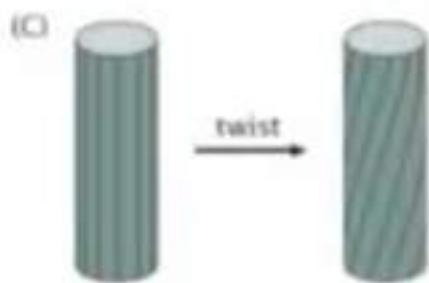
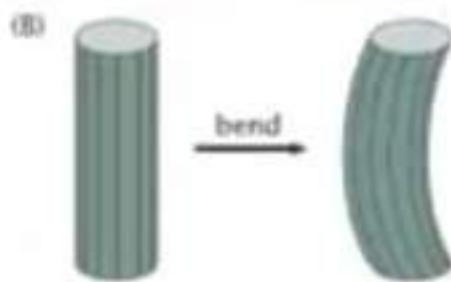
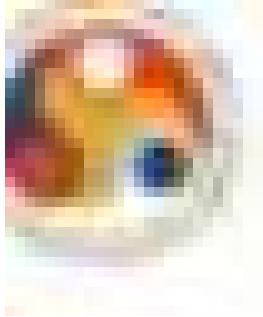


Figure from Rob Phillips:
Physical Cell Biology





Stretching (Changing Area) of Lipid Bilayer

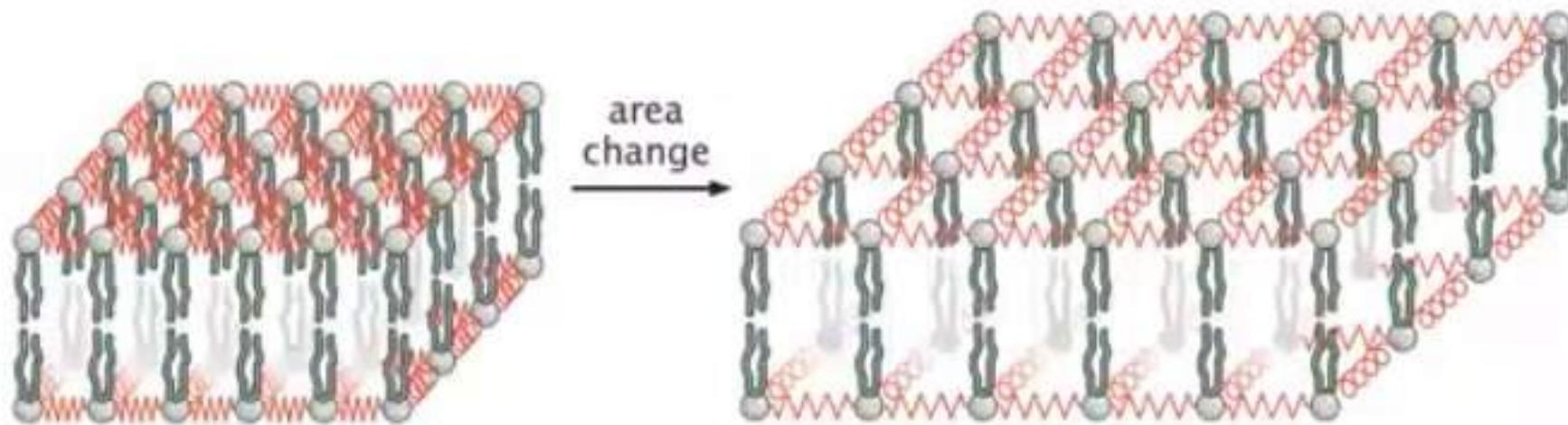


Figure 11.19: Schematic of the energy cost for stretching a membrane. When the area of the membrane is changed from its equilibrium value, there is an energetic cost.

Energetics of Beam Stretching

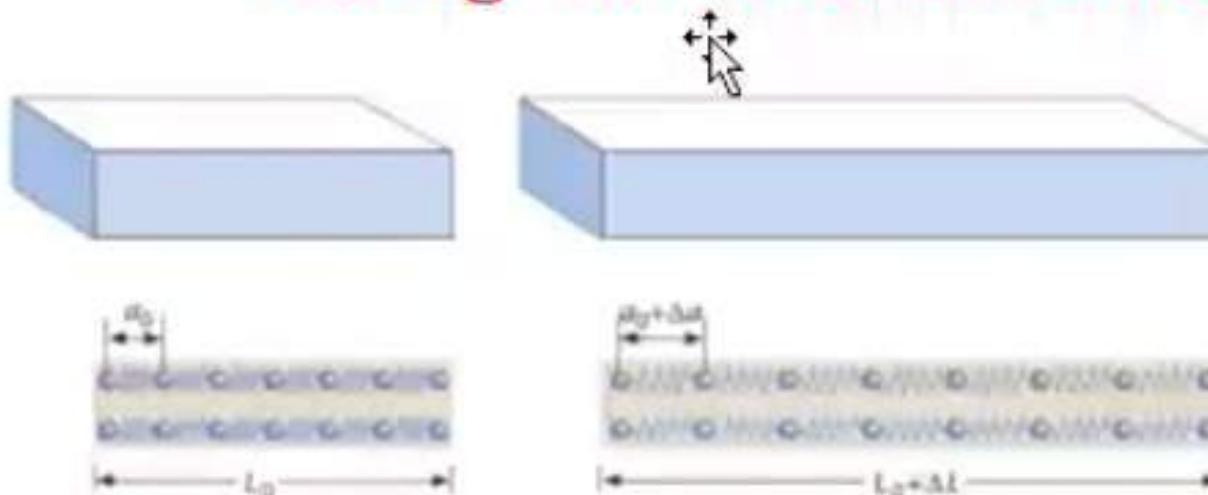


Figure 5.23: Energetics of beam stretching. The continuum description of the beam as a deformable solid can be interpreted in terms of the stretching of the individual atomic bonds.

$$\varepsilon = \frac{\Delta L}{L}.$$

Figure 5.23 suggests that we can think of the overall macroscopic deformation as imposing the stretching of a huge set of microscopic springs that correspond to the bonds between the atoms making up that beam.

$$F = -k\Delta a, \quad \text{Hooke's Law} \quad (5.24)$$

where k is the spring constant, Δa is the extension of the spring, and F is the force it engenders. Macroscopically, this same idea is written as

$$\text{Stress } \longleftarrow \frac{F}{A} = E \frac{\Delta L}{L}, \quad (5.25)$$

where F is the applied force, A is the cross-sectional area of the beam, and E is a material property known as the Young modulus, which reflects the stiffness of the beam.



ENERGY-Characterize Deformation

II Characterize Deformation of Elastic Material is energy.

Store energy in spring
due to displacement

by distance Δx
Simple idea

Once an elastic material has sufficient extensional strain

$$\text{Energy} = \frac{1}{2} k (\Delta x)^2$$

k = spring constant

Potential energy
(measured as square
of displacement Δx)

For each volume element we
compute strain energy density

We sum (integrate) over all material
elements to get total strain energy

$$\text{Energy} = \frac{EA}{2} \int \left(\frac{\Delta L}{L} \right)^2 dV$$

• \downarrow

strain energy

where A is cross sectional area of beam.

Figure from Rob Phillips: Physical Cell Biology

The concept of springiness arises from the confluence of the very important mathematical idea of a Taylor series (explained in detail in "The Math Behind the Models" on p. 215) and an allied physical idea known as Hooke's law (the same Hooke who ushered in the use of microscopy in biology and coined the term "cell"). These ideas will be developed in detail in Chapter 5 and for now we content ourselves with the conceptual framework. The fundamental mathematical idea shared by all "springs" is that the potential energy for almost any system subjected to small displacements from equilibrium is well approximated by a quadratic function of the displacement. Mathematically, we can write this as

$$\text{energy} = \frac{1}{2}kx^2. \quad (1.1)$$

What this equation states is that the potential energy increases as the square of the displacement x away from the equilibrium position. The "stiffness" k is a measure of how costly it is to move away from equilibrium and reflects the material properties of the spring itself.

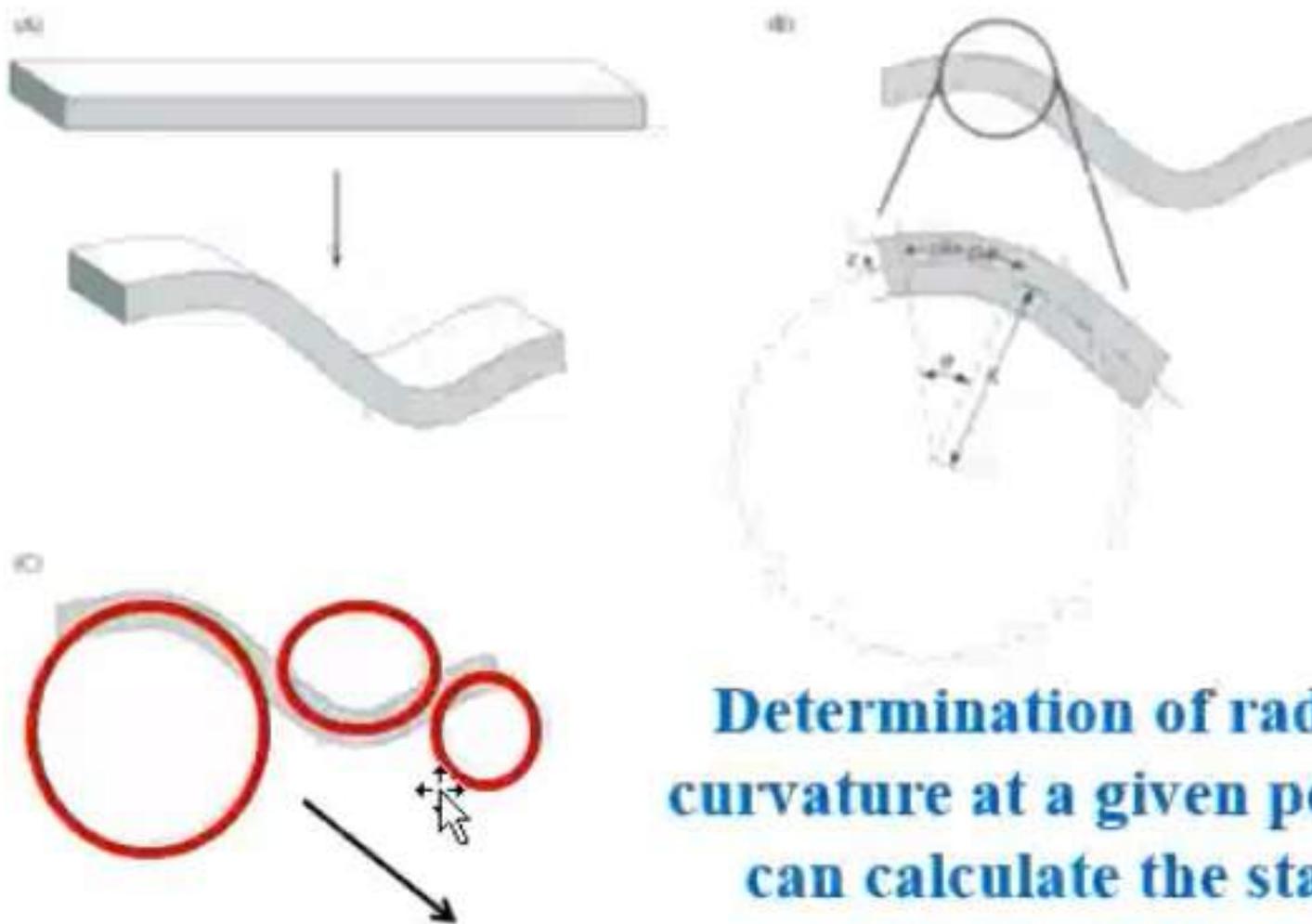
Another way of thinking about springs is that they are characterized by a restoring force that is proportional to how far the spring has been displaced from its equilibrium position. Mathematically, this idea is embodied in the equation

$$F = -kx, \quad (1.2)$$

where F is the restoring force, x is the displacement of the spring from its equilibrium position, and k is the so-called spring constant (the stiffness). The minus sign signals that the force is a restoring one towards $x = 0$, which designates the equilibrium position. This result is known as Hooke's law. When stated in this way, this kind of phys-



A Bent Beam as Collection of Stretched Beams



Determination of radius of curvature at a given point, we can calculate the state of strain/energy

Collection of Segments each of which is bent locally into an arc

Figure from Rob Phillips: Physical Cell Biology



CYTOSKELETON (NETWORK OF BEAMS)





CYTOSKELETON AND BEAM THEORY

- Cytoskeleton is a collection of Elastic Beams.
- Determine mechanical properties of cell.
- Stiffness of structures depends
 - ❖ Intrinsic flexibility of filaments
 - ❖ Presence/absence of filament binding proteins.
 - ❖ Large scale organization of filaments into bundles.

CYTOSKELETON (NETWORK OF BEAMS)

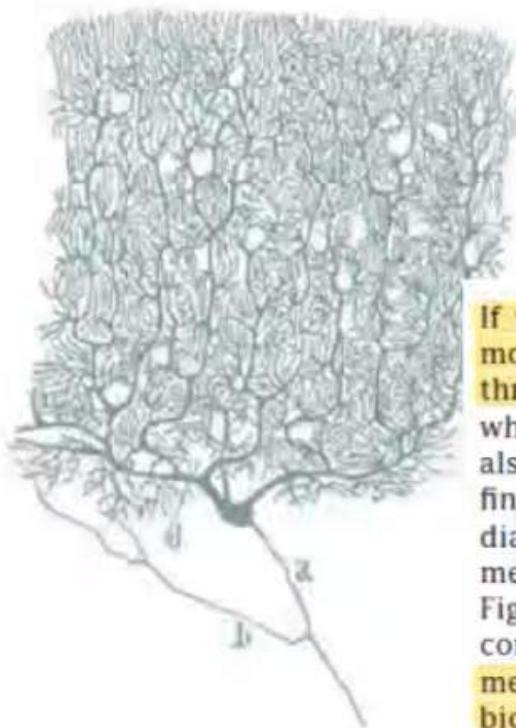
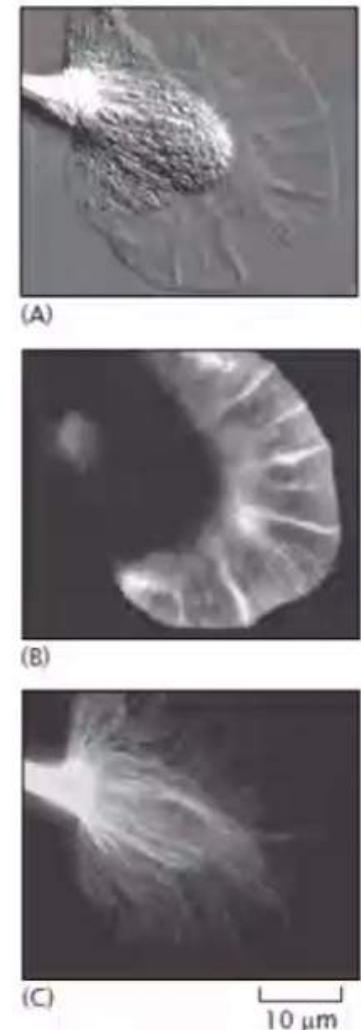


Figure 10.26: Drawing of a neuron. This figure shows one of the many impressive drawings from Ramón y Cajal of a neuron. In particular, this figure shows a human Purkinje cell and illustrates the morphological complexity dictated by cytoskeletal proteins. (Adapted from S. Ramón y Cajal, *Histology of the Nervous System*. Oxford University Press, 1995.)

If we take the neuron shown in Figure 10.26, and separate out the molecularly distinct filament types that it contains, we will find that three predominate. One group of these filaments are microtubules, which tend to be straight filaments about 25 nm in diameter. We will also find actin filaments, which are roughly 8 nm in diameter, and finally, intermediate filaments, so-called because they are intermediate in size between actin and microtubules. These different filaments have distinct locations within the neuronal cell as depicted in Figure 10.27, which shows actin and microtubules within the growth cone found at the tip of a developing neuron. The various filaments also have different mechanical properties and perform distinct biological functions.

Figure 10.27: Cytoskeletal filaments in a neural growth cone. (A) Differential interference contrast image of an *Aplysia* (sea slug) neuron growth cone. (B) Fluorescence image of actin in the growth cone. (C) Fluorescence image of microtubules in the growth cone. The distribution of intermediate filaments (neurofilaments) is similar to the distribution of microtubules shown here. (Courtesy of P. Forscher.)





MICROTUBULES?

MICROTUBULES

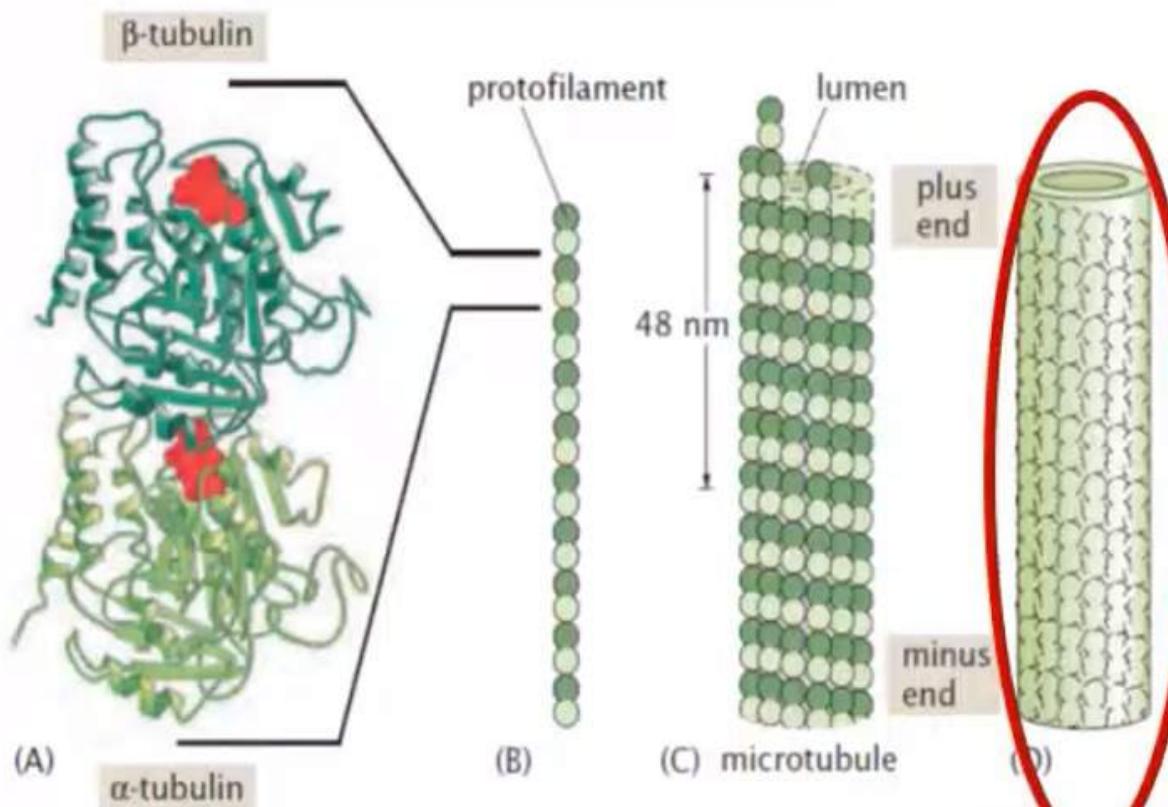


Figure 10.28: Structure of a microtubule. (A) Ribbon diagram depiction of a tubulin subunit, consisting of a dimer of the two proteins α -tubulin and β -tubulin. Bound GTP molecules are shown in red. (B) Individual tubulin subunits assemble in a head-to-tail fashion. A single string of subunits is called a protofilament (such structures are unstable on their own). (C) Microtubules are hollow cylinders of 13 protofilaments. Because of the intrinsic polarity of the tubulin subunit, the microtubule itself is also polarized, with all β -tubulin proteins exposed at the plus end and all α -tubulin proteins exposed at the minus end. (D) For idealized representations, we will treat the microtubule as a uniform hollow cylinder with a diameter of 25 nm. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. Garland Science, 2008.)

Implicated in cellular processes and help in motion of molecular motors like kinesin and dynein

Microtubule as cylinder



Figure from Rob Phillips: Physical Cell Biology

MICROTUBULES VIEWED USING MICROSCOPY

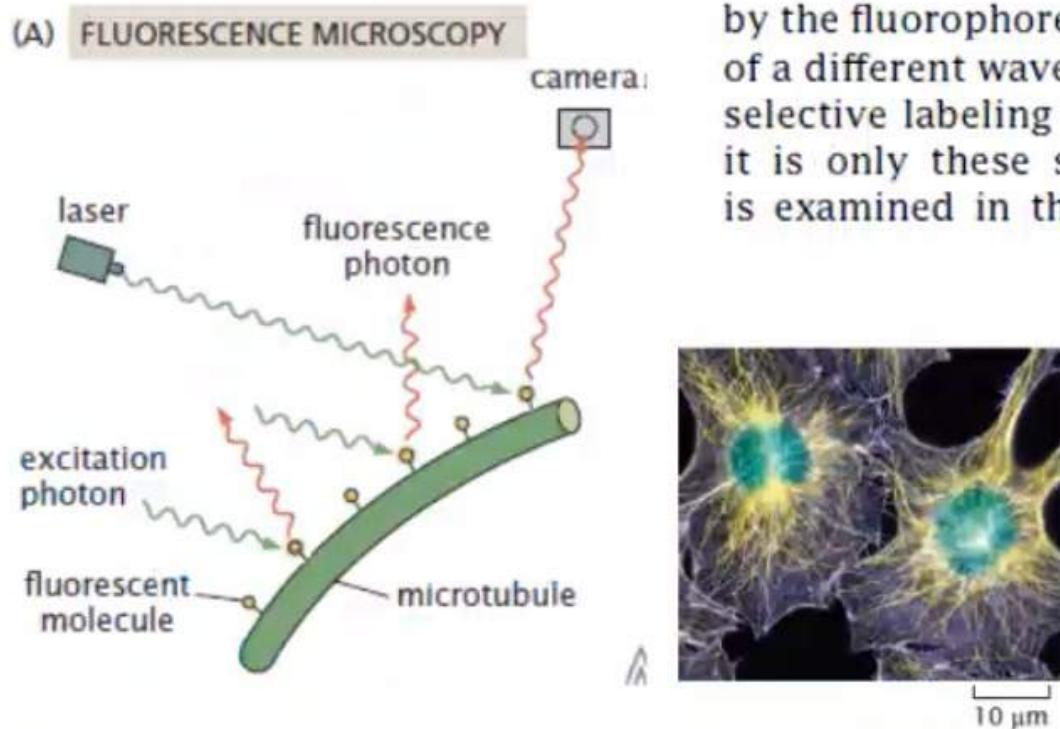


Figure 2.13(A) shows a schematic of the way in which light can excite fluorescence in samples that have some distribution of fluorescent molecules within them. In particular, this example shows a schematic of a microtubule that has some distribution of fluorophores along its length. Incident photons of one wavelength are absorbed by the fluorophores and this excitation leads them to emit light of a different wavelength, which is then detected. As a result of selective labeling of only the microtubules with fluorophores, it is only these structures that are observed when the cell is examined in the microscope.

Figure 2.13: Experimental techniques that have revealed the structure of both cells and their organelles. (A) Fluorescence microscopy and the associated image of a fibroblast with labeled microtubules (yellow) and DNA (green).



CYTOSKELETON BUCKLING



CYTOSKELETON BUCKLING

- From a practical perspective (push and pull of beams with forces) can exceed Theory of Elasticity and need not be small
- Apart from simple deformations ‘buckling’ can occur
- Cytoskeletal filaments are exerted to compressive forces during polymerization.



SCHEMA OF BUCKLING

SCHEMA OF BUCKLING PROCESS

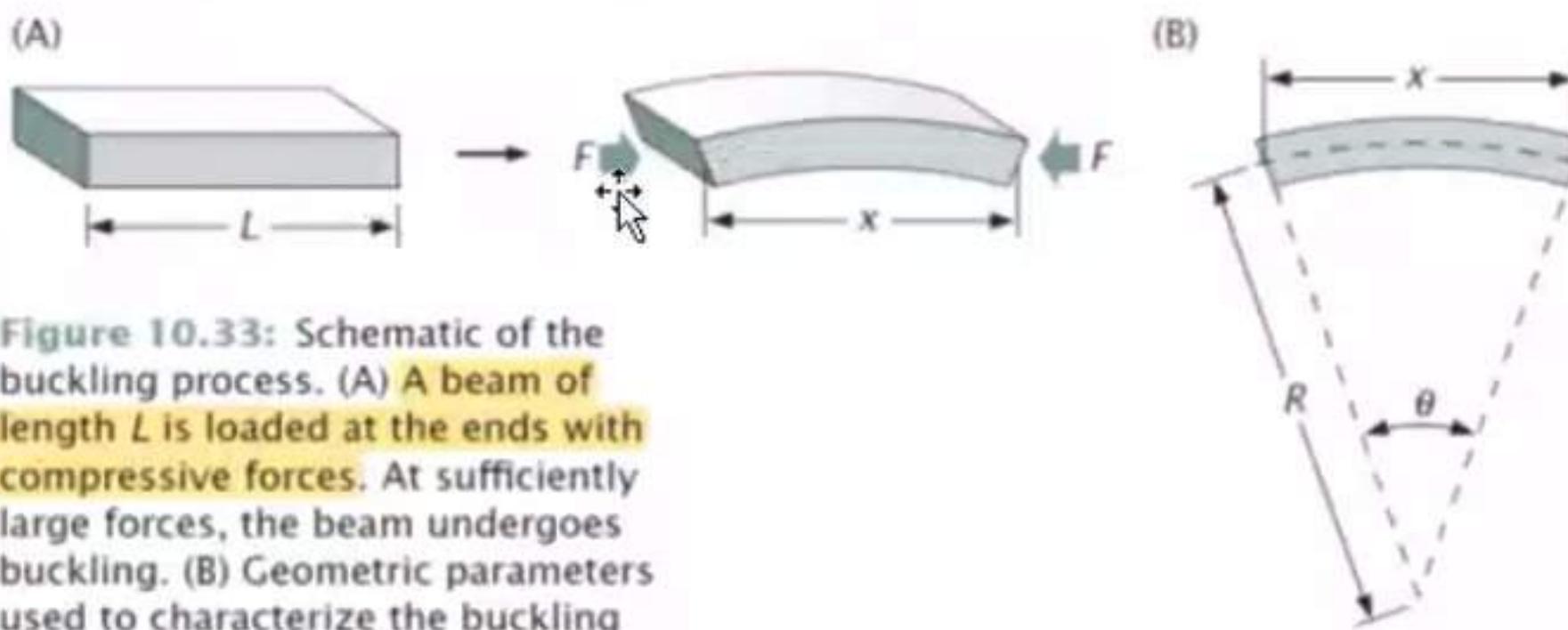


Figure 10.33: Schematic of the buckling process. (A) A beam of length L is loaded at the ends with compressive forces. At sufficiently large forces, the beam undergoes buckling. (B) Geometric parameters used to characterize the buckling process.

A simple estimate of the buckling force is determined by energetics beam deformation into a semicircular arc

Figure from Rob Phillips: Physical Cell Biology

MICROTUBULE BUCKLING DURING POLYMERIZATION

Due to thermal fluctuations cause force build up to buckle microtubule

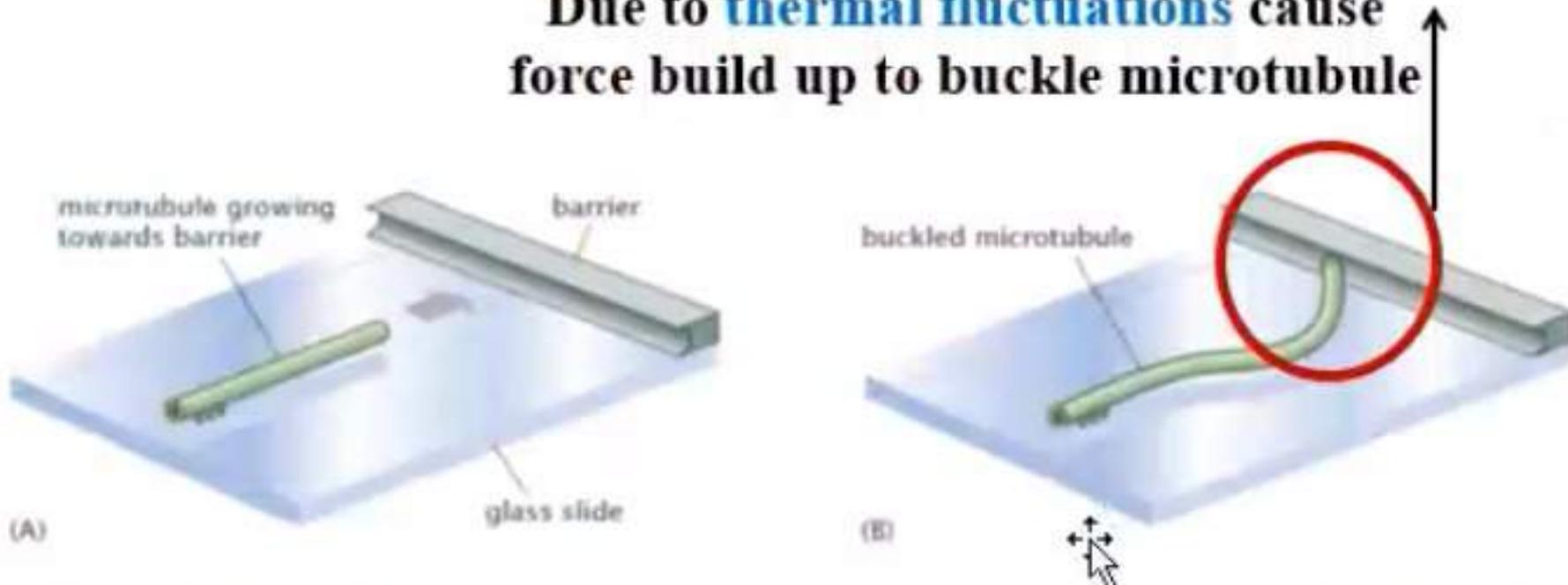


Figure 10.34: Microtubule buckling during polymerization. (A) At the start of the experiment, a microtubule is tightly bound to a glass slide, oriented such that its plus end is growing towards a barrier wall with a small overhang. With the addition of tubulin subunits, the microtubule grows freely until it contacts the wall. (B) Small thermal fluctuations in the position of the tip of the growing microtubule are thought to allow sufficient space for insertion of tubulin subunits occasionally, even when the microtubule tip is in contact with the wall. The force built up at this interface causes the microtubule to bend and then to buckle. (Adapted from M. Dogterom and B. Yurke, Science 278:856, 1997.)

Figure from Rob Phillips: Physical Cell Biology

In Short Cell is crowded

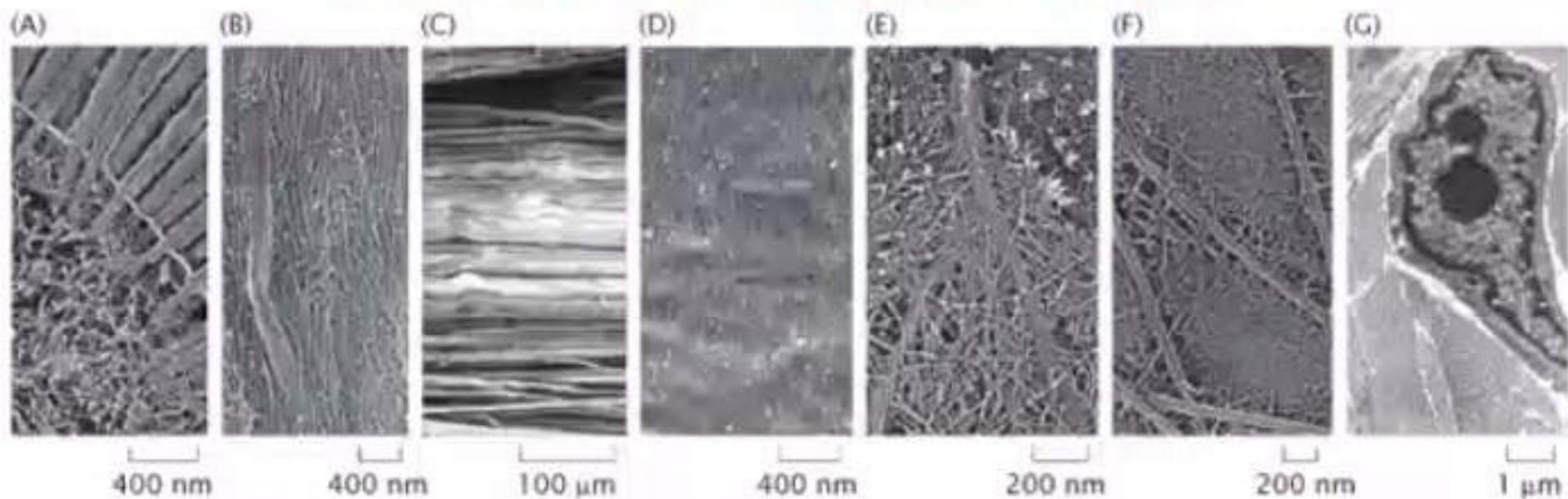
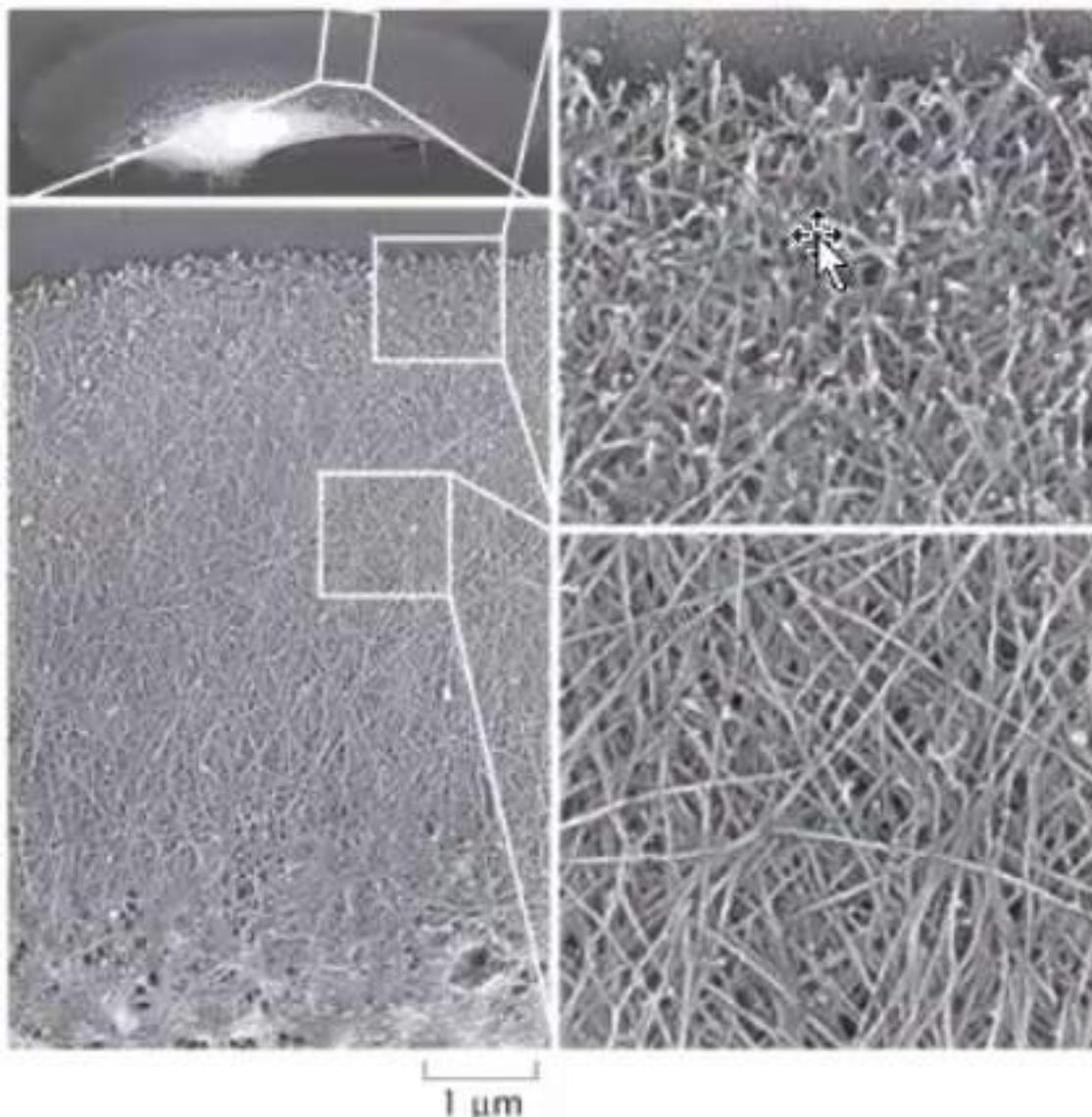


Figure 14.1 Physical Biology of the Cell, 2ed, (© Garland Science 2013)

- **Filamentous network found in cell and extracellular matrix**

Figure from Rob Phillips: Physical Cell Biology

In Short Cell is crowded



Actin Filaments

Two regions highly magnified

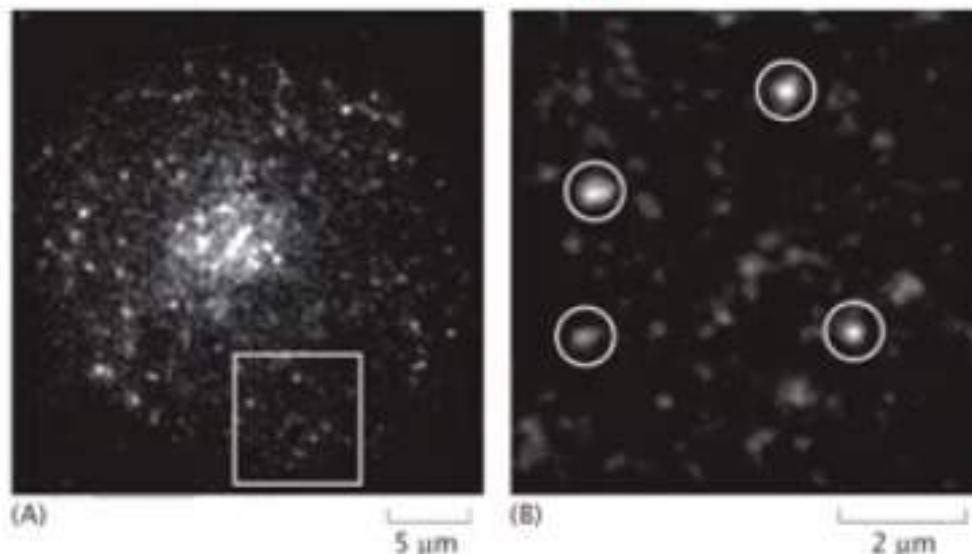
Figure 14.2 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Figure from Rob Phillips: Physical Cell Biology

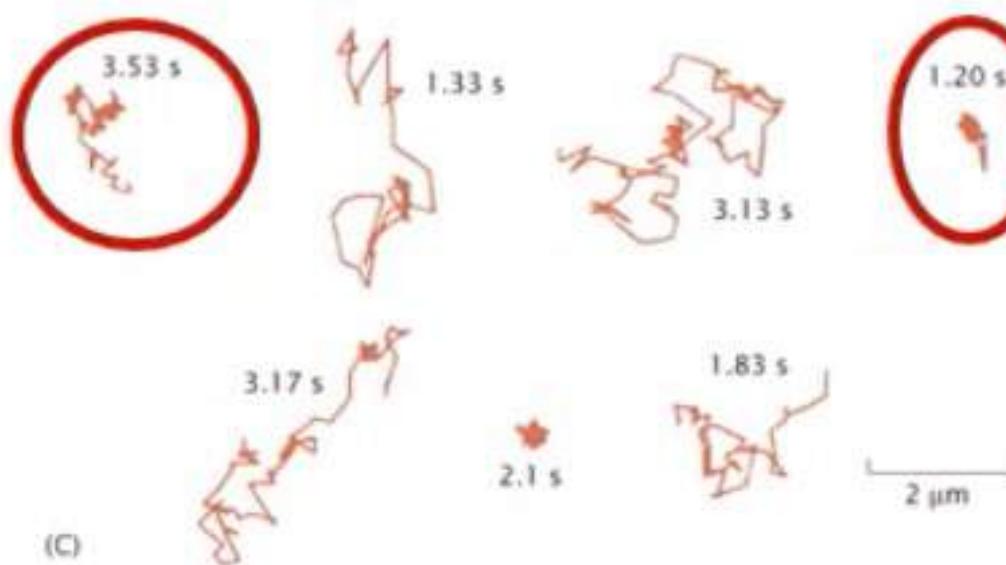


Observing Cell Crowding

Crowding alters Diffusive Dynamics



Video microscopy of membrane proteins at cell surface during free diffusion



Some molecules appear stationary (**red circles**) while other travel long distances

Figure 14.5 Physical Biology of the Cell, 2nd ed. (© Garland Science 2013)

Modified Figure from Rob Phillips: Physical Cell Biology



ANY QUESTIONS

THANKS

Todays Lecture

Membrane Transport Process

a. Neuron and Types

b. Resting Potential, Nernst Equation Expression

**Acknowledgment: Aggregated Lecture from lot of resources
(Textbooks/Coursera/Youtube lectures, Journal Papers)**



Fundamental Unit of Nervous system

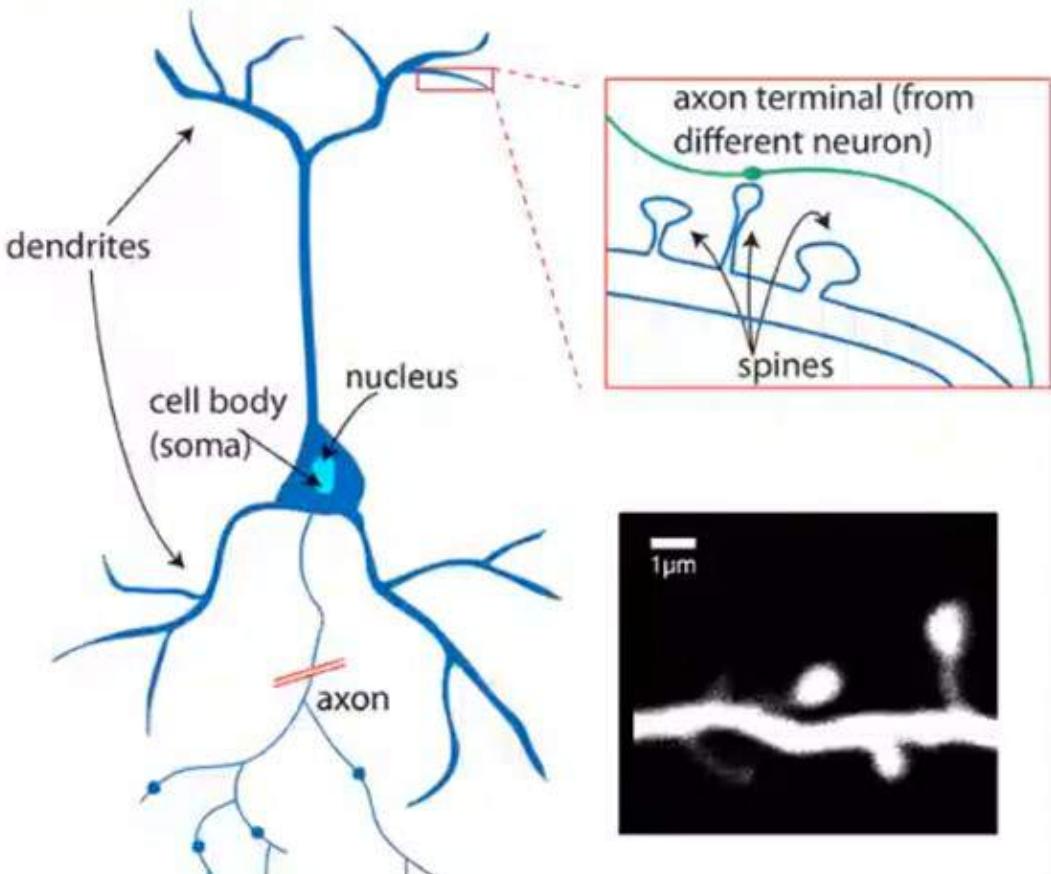


Neurons:

- a) Receives sensory input from external world and sends commands to our muscles
- b) Specialized type of cell for information transfer
- c) Fires action potential (AP).

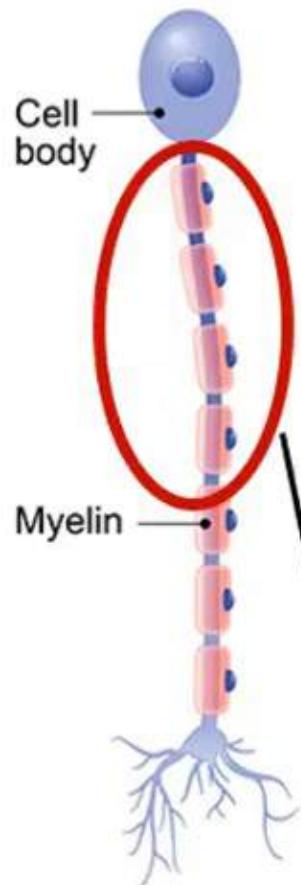
Structure of Neuron

- ❖ *Dendrites*: Receive signals from neighboring neurons (like tree branch).
- ❖ *Axon*: Transmit Signals to other neurons (like tree roots)
- ❖ *Soma*: Has nucleus with DNA where proteins are made (like tree trunk)
- ❖ *Myelin Sheath*: Speeds up signal transmission along axon (like bark of tree)



Neuron Types based on Structure

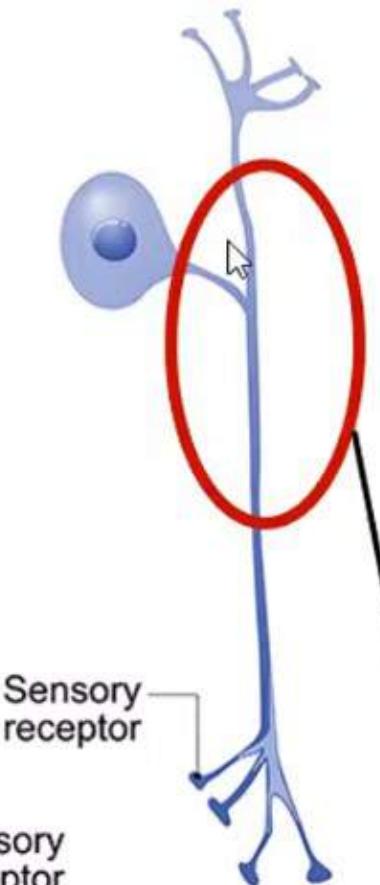
Unipolar



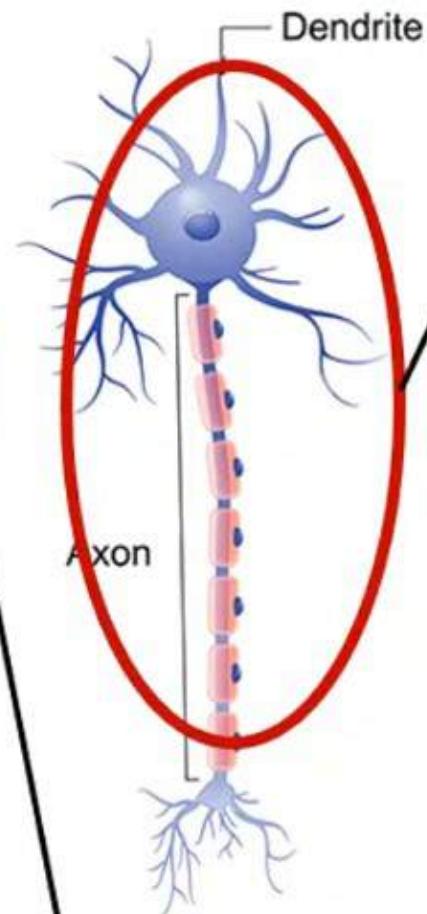
Bipolar



Pseudounipolar



Multipolar



Multiple processes
from cell body
(Dendrites and
Axon)

One process called Neurite(Axon or dendrite) from cell body

Axon is split from cell body

Neurons forming Complex Network?

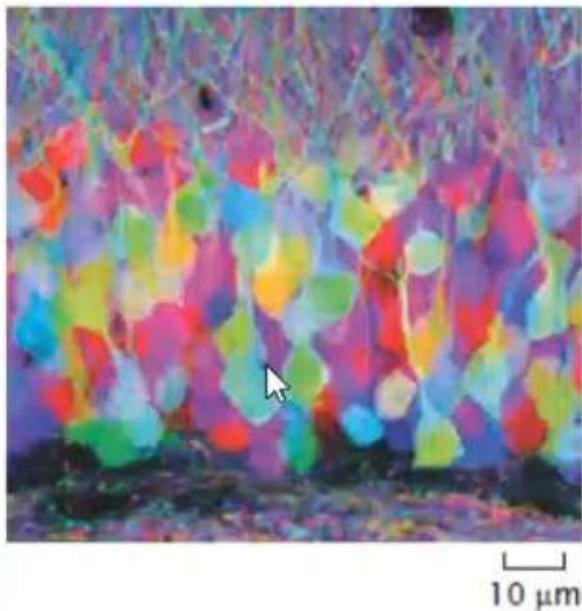
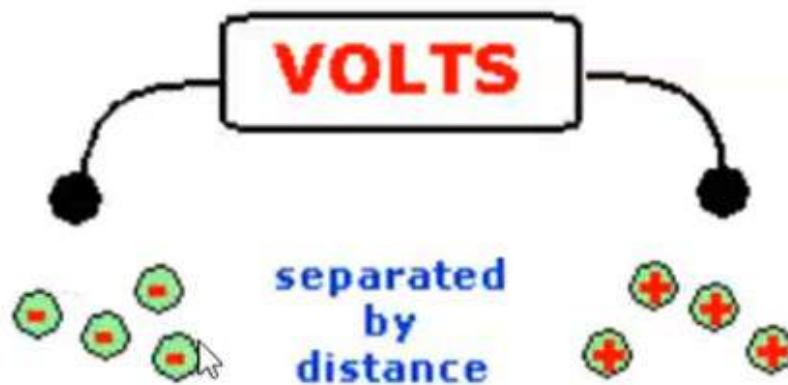


Figure 2.42: Illustration of a complex network of cells formed by neurons. Using multiple fluorophores simultaneously to create a BrainBow, the structure of the nerves is elucidated. (Adapted from J. W. Lichtman et al., *Nat. Rev. Neurosci.* 9:417, 2008.)

An example of a collection of fluorescently labeled neurons is shown in Figure 2.42. Note that the branches (dendrites and axons) that reach out from the various cells have lengths far in excess of the $10\text{ }\mu\text{m}$ scale characteristic of typical eukaryotes. Indeed, axons of some neurons can have lengths of centimeters and more.

Electric Potential difference



- **Electrical potential difference** exists when there is net separation of charges between two locations. Unit is **Volt**



17.2.1 The Electrical Status of Cells and Their Membranes

In our everyday macroscopic encounters with electricity, we are used to thinking of electrons traveling down metallic wires with a flow of negative charge. However, movement of ions, including those with positive charge, can also produce electrical currents and potential differences. Indeed, the earliest batteries invented by Volta and Daniell in the late eighteenth and early nineteenth centuries relied on current carried by ions such as Zn^{2+} , Cu^{2+} , and Ag^+ ions. In cells, in a few cases, electron transport is used directly to produce charge separation, as in photosynthesis in chloroplasts and electron transport in mitochondria. More commonly though, cells create and manipulate transmembrane gradients of positive ions, the most important being Na^+ , K^+ , and Ca^{2+} ions.

Permeability of Cell Membrane

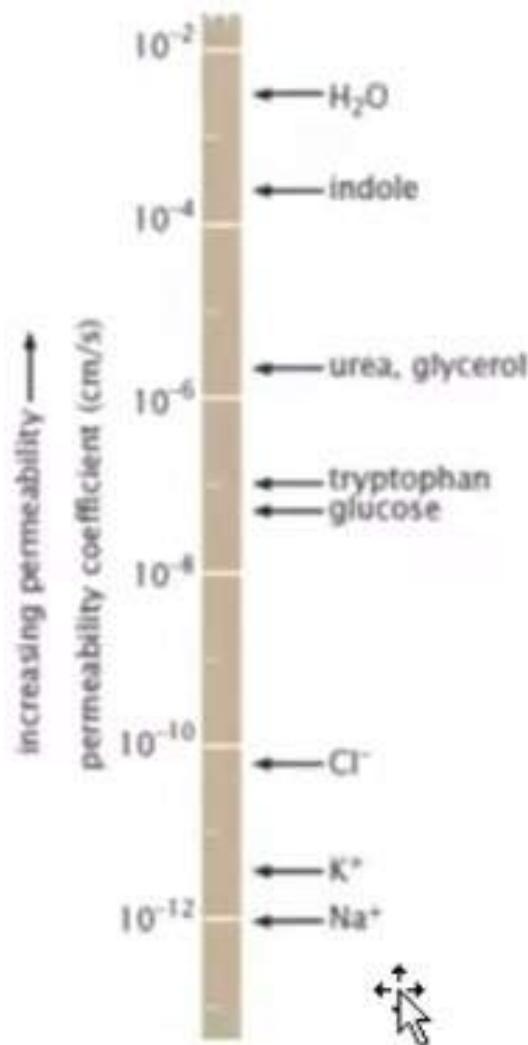
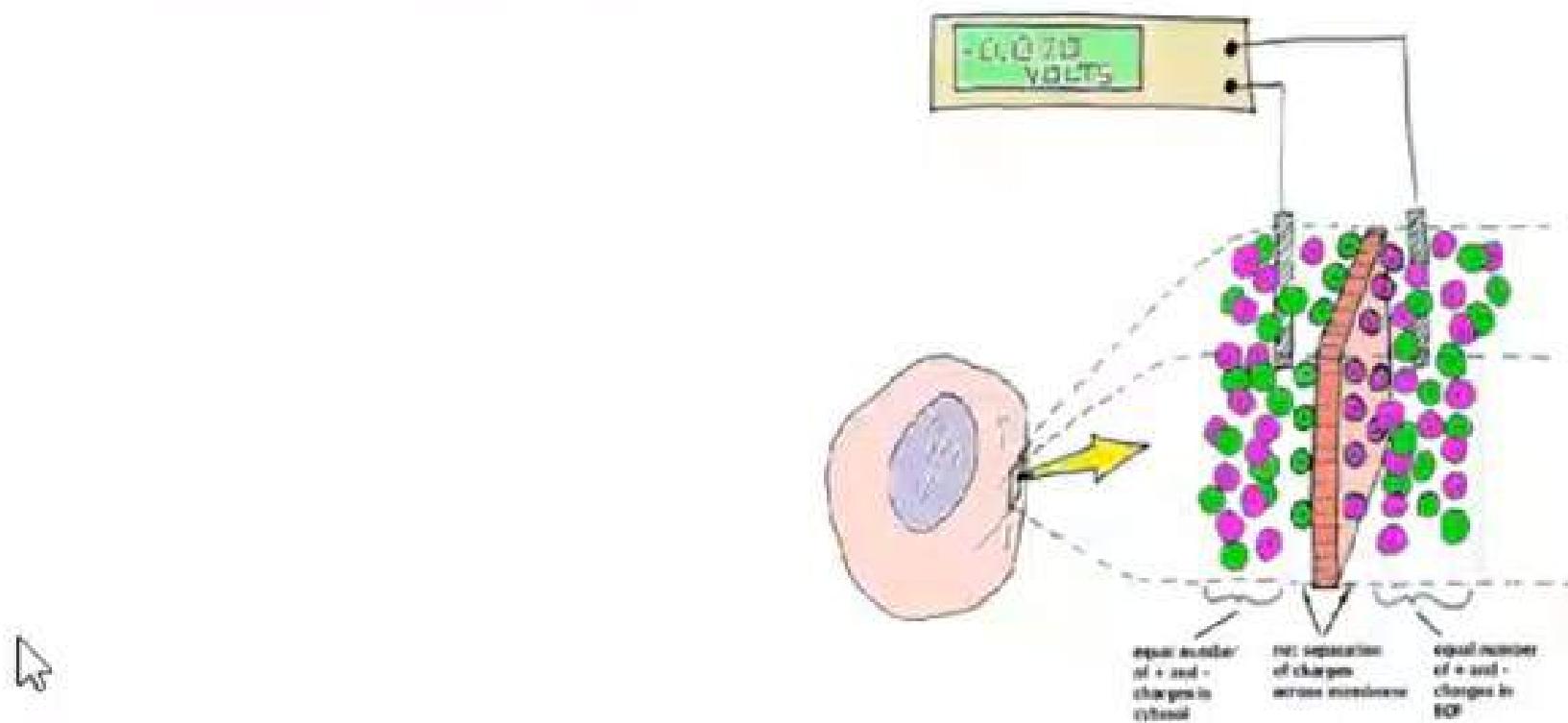


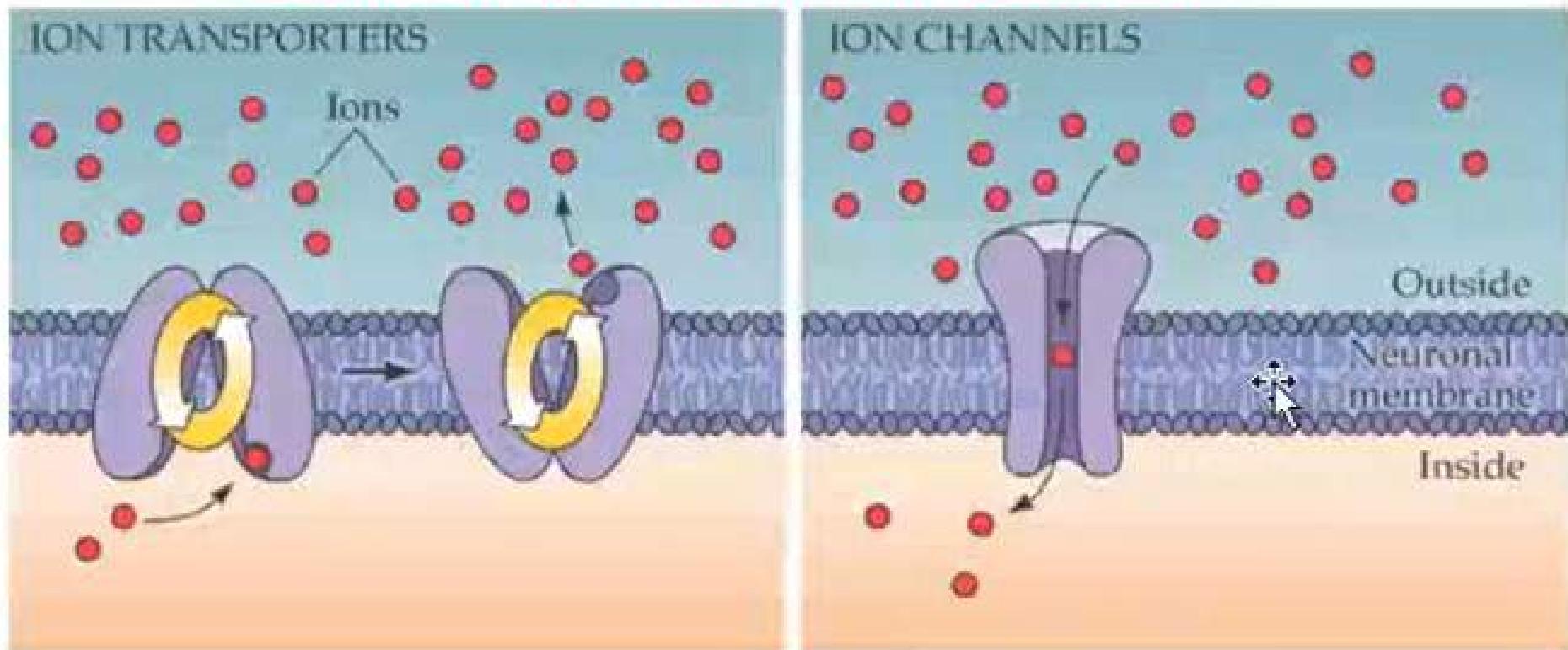
Figure 11.11: Range of measured membrane permeabilities for different molecular species. **Membranes are most permeable to water and least permeable to ions.** (Adapted from R. N. Robertson, *The Lively Membranes*. Cambridge. University Press, 1983.)

Resting Membrane Potential in neurons

- When a nerve or muscle cell is at "rest", its membrane potential is called the **Resting membrane potential**.
- Changes in their membrane potentials are used to code and transmit information across neurons.



Resting membrane potential: Channels in neuronal membrane



Ion Transporters: Select one ion type and moves them inside or outside of the cell (creating concentration gradient)

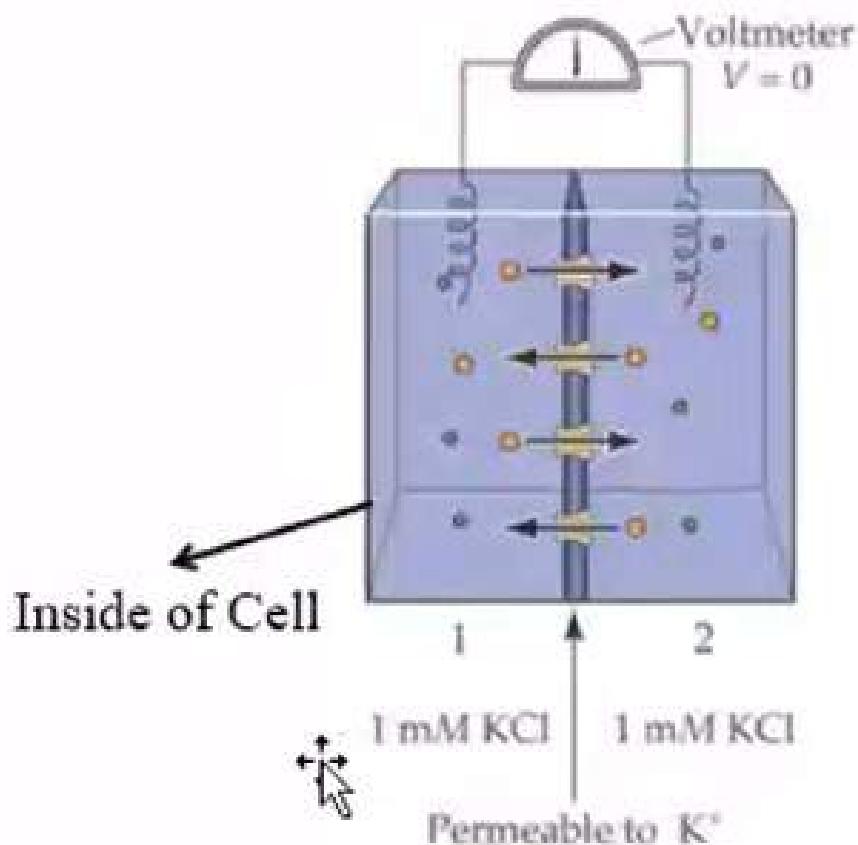
Ion Channels: Can allow any or specific ions

:

Resting membrane potential in Neurons

- Conditions for resting membrane potential:
 - 1) Concentration gradient → Differing concentrations between inside/outside of cell for ions leads to a gradient
 - 2) Semi-permeable membrane → Ions needs to flow. Ion channels are selective open to one ion type
- Role of active transporters:
 - 1) None! → Not involved in creating Resting membrane potential which is a passive process
 - 2) They establish the concentration gradient

No Concentration Gradient; Resting membrane potential = 0



Assume the neuron cell membrane is packed into a compact box and connected to Voltmeter

Figure from book: Neuroscience by Dale Perves et al

But in Reality for Humans and Squids

Measured ion concentrations

TABLE 2.1

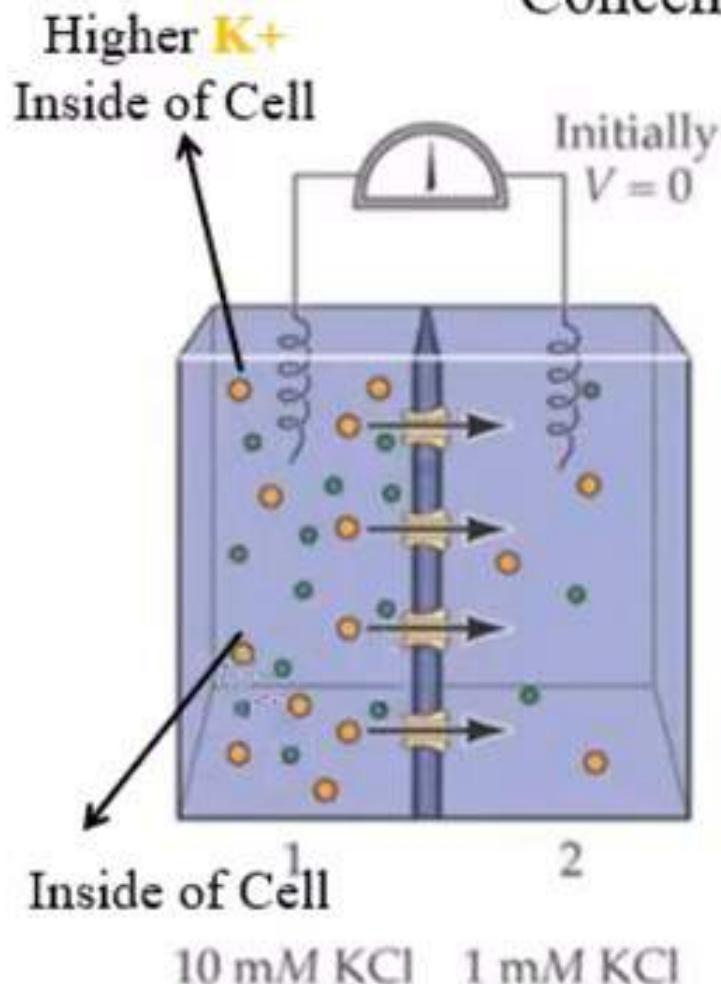
Extracellular and Intracellular Ion Concentrations

Ion	Concentration (mM)	
	Intracellular	Extracellular
Squid neuron		
Potassium (K^+)	400	20
Sodium (Na^+)	50	440
Chloride (Cl^-)	40–150	560
Calcium (Ca^{2+})	0.0001	10
Mammalian neuron		
Potassium (K^+)	140	5
Sodium (Na^+)	5–15	145
Chloride (Cl^-)	4–30	110
Calcium (Ca^{2+})	0.0001	1–2

Figure from book: Neuroscience by Dale Perves et al

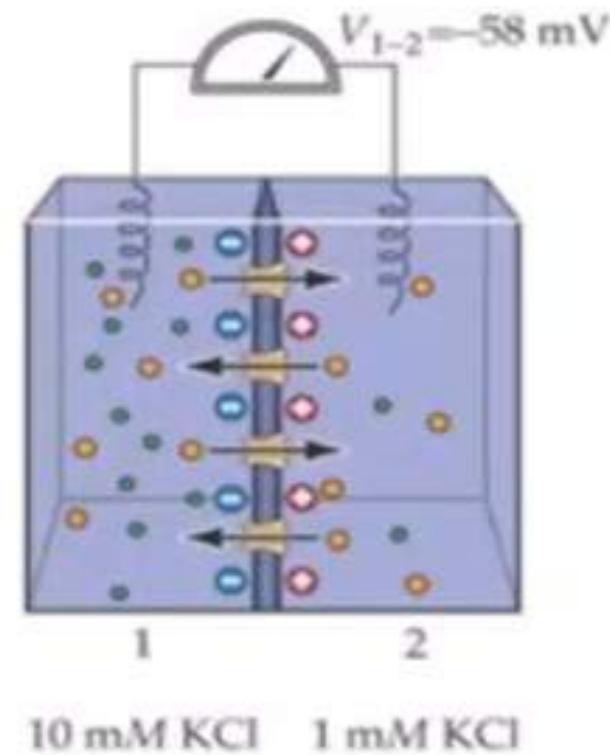
But in Reality for Humans and Squids

Semi permeable membrane (specific to K⁺) with
Concentration Gradient



Diffusion force
equals
electrophoretic
force

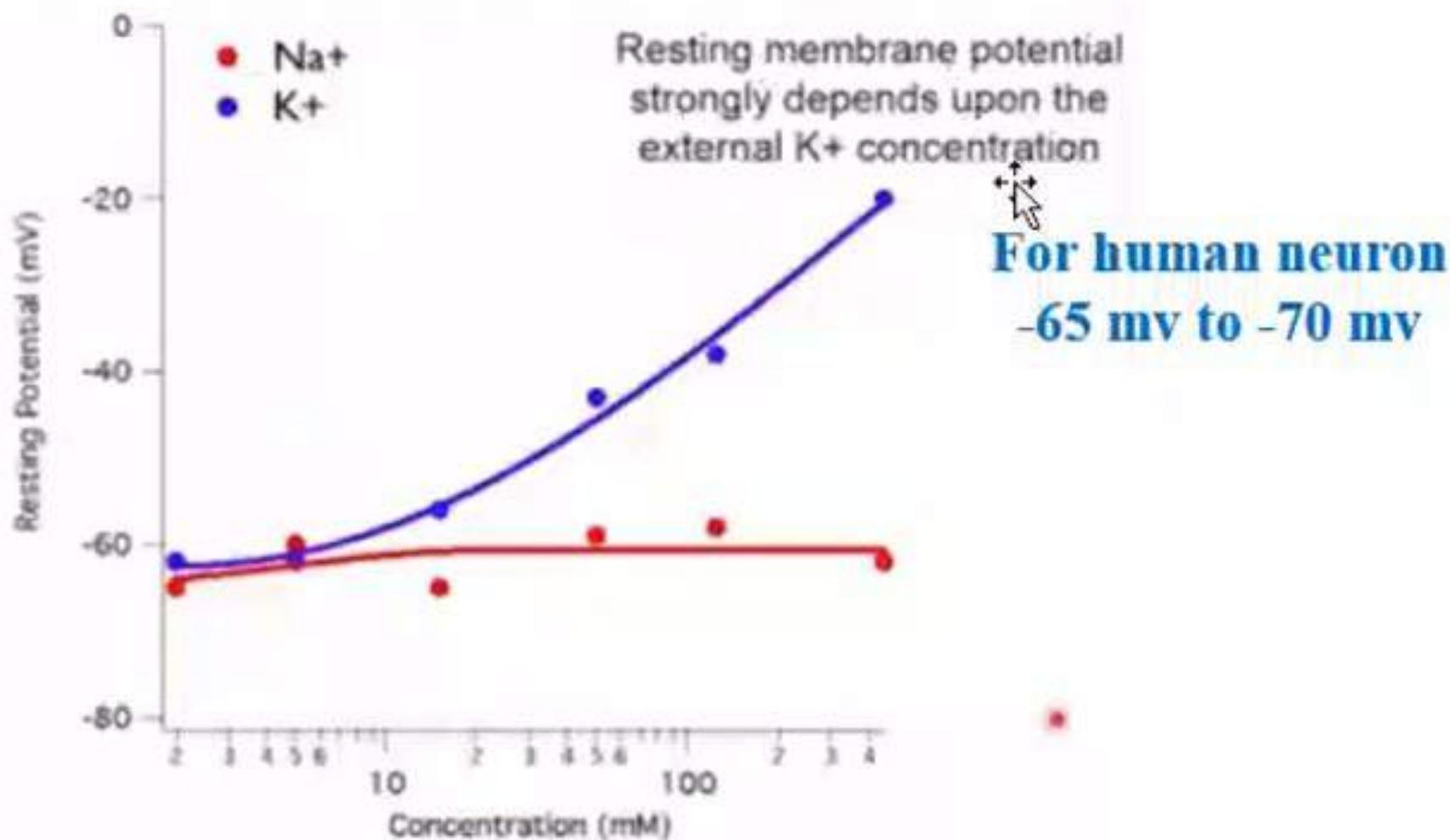
→ At equilibrium



For squid
neuron
 $(R_p) = -58 \text{ mv}$

Figure from book: Neuroscience by Dale Perves et al

Resting Membrane Potential (Important Ion)



The membrane of human neurons is much more permeable to K^+ than to Na^+ , so the resting membrane potential is close to the **equilibrium potential K^+**

Resting membrane potential
(If membrane permeable to Sodium)
Then Resting potential^I=20mv

Resting Membrane Potential in terms of Electrical components

The combined electrochemical properties of the lipid bilayer, channels, and pumps provide a patch of membrane with electrical properties equivalent to a set of resistors (which are voltage-dependent), batteries (whose voltage is set by the ion concentration difference), and a capacitor, all connected in parallel. By selectively opening and closing transmembrane ion channels, which can specifically conduct only a subset of ions (usually only a single type of ion), the cell can tune its membrane electrical potential. This process is at the heart of electrical signaling in excitable cells.

Resting Membrane Potential in neurons



Julius Bernstein
German scientist

Bernstein (1902, 1912) correctly proposed that excitable cells are surrounded by a membrane selectively permeable to K^+ ions at rest and that during excitation the membrane "membrane breakdown" to be permeable to other ions.

Resting Membrane Potential

Resting Membrane Potential is developed in each and every neuronal cell (brain or muscle)

In each neuron (say 100 billion in brain) this entire process happens passively and no energy is consumed.

I

Hence when relaxing we consume very less energy.

NERNST POTENTIAL/ION CONCENTRATIONS for Mammalian Skeletal muscle

Table 17.1: Ion concentrations and the Nernst potential for small ions within the cell. The numbers are typical of mammalian skeletal muscle cells, which have a resting potential of $V_{mem} = -90$ mV. (Data adapted from B. Hille, Ion Channels of Excitable Membranes. Sinauer Associates, 2001.)

Ion species	Intracellular concentration (mM)	Extracellular concentration (mM)	Nernst potential (mV)
K ⁺	155	4	-98
Na ⁺	12	145	67
Ca ²⁺	10^{-4}	1.5	130
Cl ⁻	4	120	-90

NERNST EQUATION

Mathematical eqn for resting potential for any particular ion concentration and membrane permeability

Molar flux from diffusion

Potassium Flux
from diffusion

$$M_d = -D \frac{dc}{dn}$$

diffusion coefficient

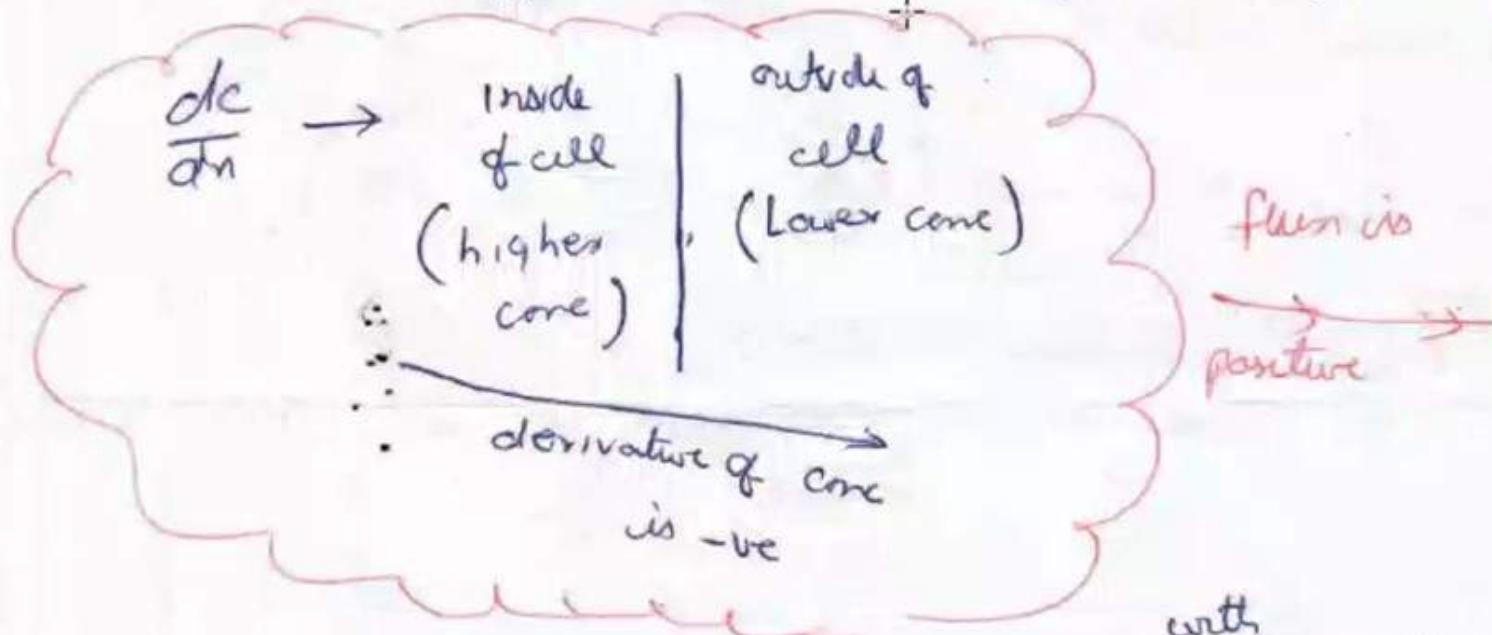
c = concentration of specific ion

n = distance across the membrane.

M_d = Molar flux from diffusion

inside | outside

M_d = Molar flux from diffusion



D = diffusion coefficient (ease with the ions diffuse), with

Molar flux from electrophoretic effect

D = diffusion coefficient (ease with the ion is diffuse).

Molar flux from electrophoretic effect

$$M_e = -\mu C \frac{d\psi}{dn}$$

μ = ion mobility in medium

ψ = local potential

M_e = Molar flux from electrophoretic effect for Potassium

Total Flux (M_k^+)

$$M_k^+ = M_d + M_e = -D \frac{dc}{dn} - \mu C \frac{d\psi}{dn}$$

↳ Total molar flux for Potassium

We are not interested in flux but we want electrical

Relationship between flux and current]

$$I = M_k z F = -zF \left(D \frac{dc}{dn} + \cancel{\mu c} \frac{d\delta F}{dn} \right) \text{ coulombs/sec.}$$

I = electric current

z = Valence of ion of interest (ions which are positively charged and also negatively).

F = Faraday constant

Convert mobility into variables already present in Eqn.

$$\mu = \frac{zFD}{RT}$$

Using Einstein's relationship

$$R = g$$



R = gas constant, T = absolute temperature

$$I = M_{K^+} + ZF = -ZF \left(\frac{Ddc}{dn} + \frac{DZF}{RT} c \frac{d\psi}{dn} \right).$$

At Equilibrium $I = 0$

$$\int_{\text{outside}}^{\text{inside}} \frac{d\psi}{dn} = -\frac{RT}{ZF} \int_{\text{outside}}^{\text{inside}} \frac{1}{c} \frac{dc}{dn}$$

Now integrating across membrane

$$\psi_{\text{inside}} - \psi_{\text{outside}} = -\frac{RT}{ZF} \ln \frac{[K^+]_{\text{inside}}}{[K^+]_{\text{outside}}}$$

$$V_m = \frac{RT}{ZF} \ln \frac{[K^+]_{\text{outside}}}{[K^+]_{\text{inside}}}$$

$$\int \frac{dn}{\text{outside}}$$

$$zF \int \frac{c}{\text{outside}} dn$$

Now integrating across membrane

$$\psi_{\text{inside}} - \psi_{\text{outside}} = \frac{-RT}{zF} \ln \frac{[K^+]_{\text{inside}}}{[K^+]_{\text{outside}}}$$

$$V_m = \frac{RT}{zF} \ln \frac{[K^+]_{\text{outside}}}{[K^+]_{\text{inside}}} +$$

R = universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$)

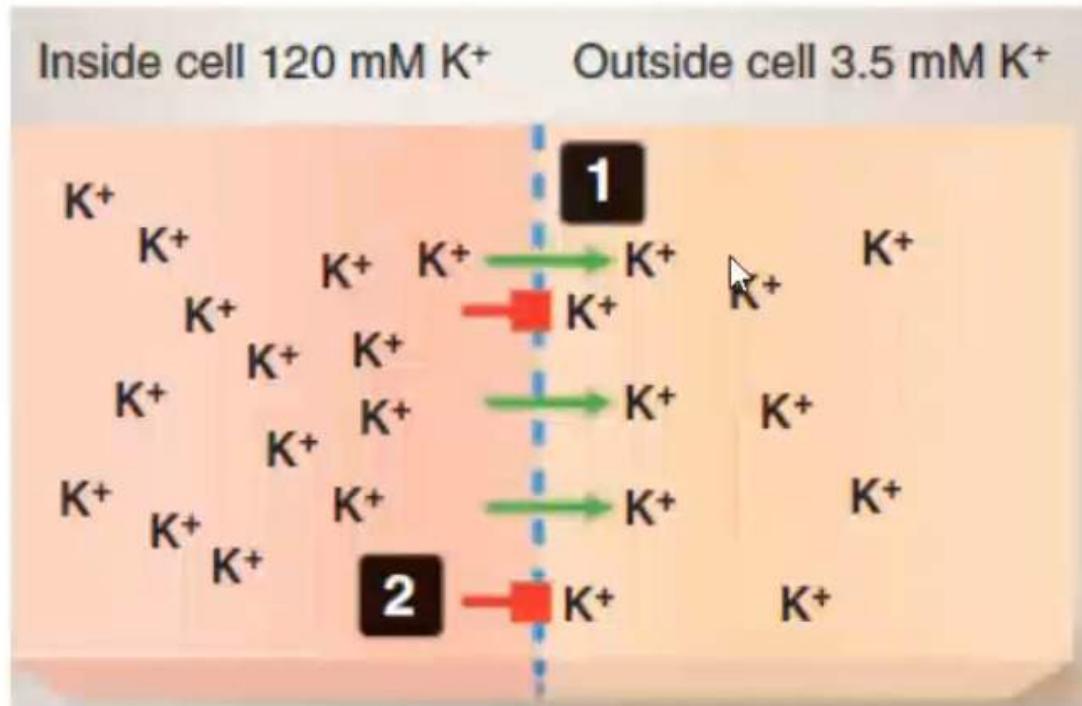
T = Temperature in Kelvin

F = Faraday's constant 96485 C mol^{-1}

$$V_m = \frac{RT}{zF} \ln \frac{[n]_{\text{outside}}}{[n]_{\text{inside}}}$$

$[n]$ = total concentration of
ions \approx

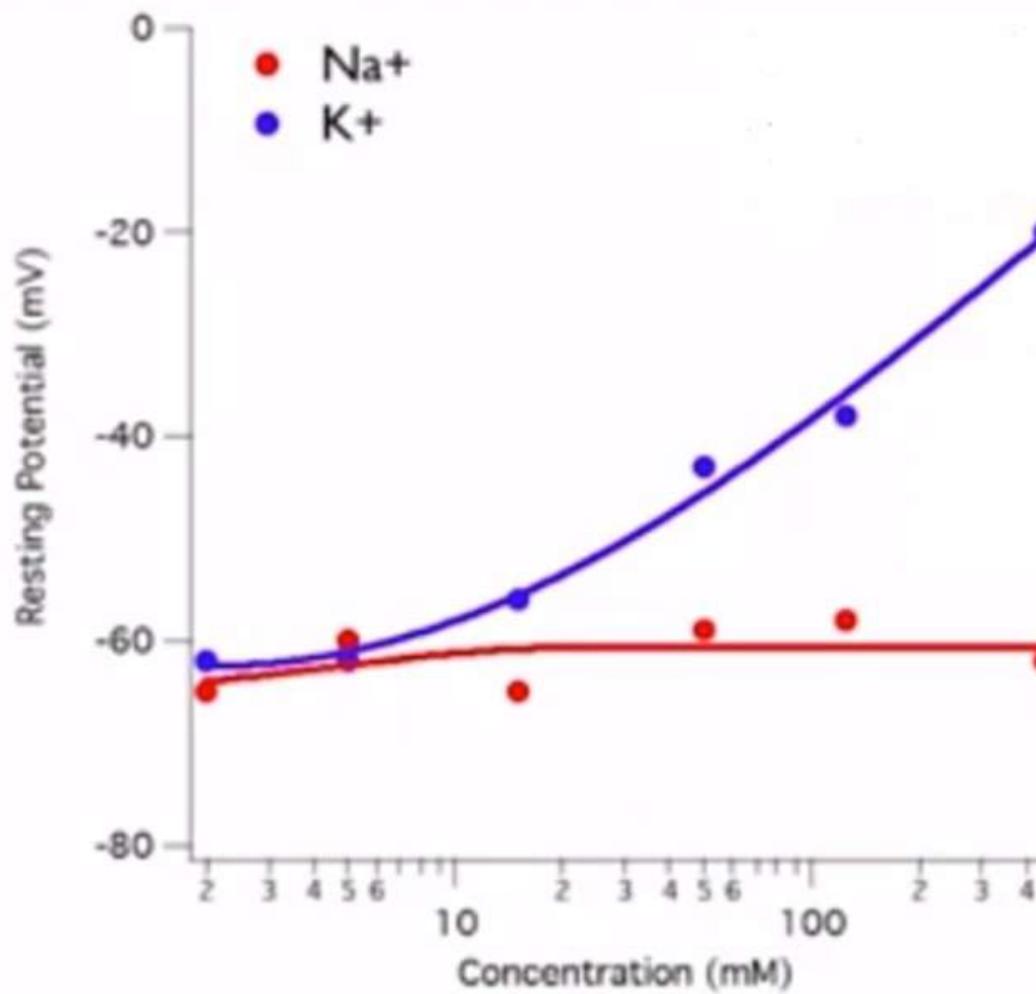
Recap-Nernst Eqn



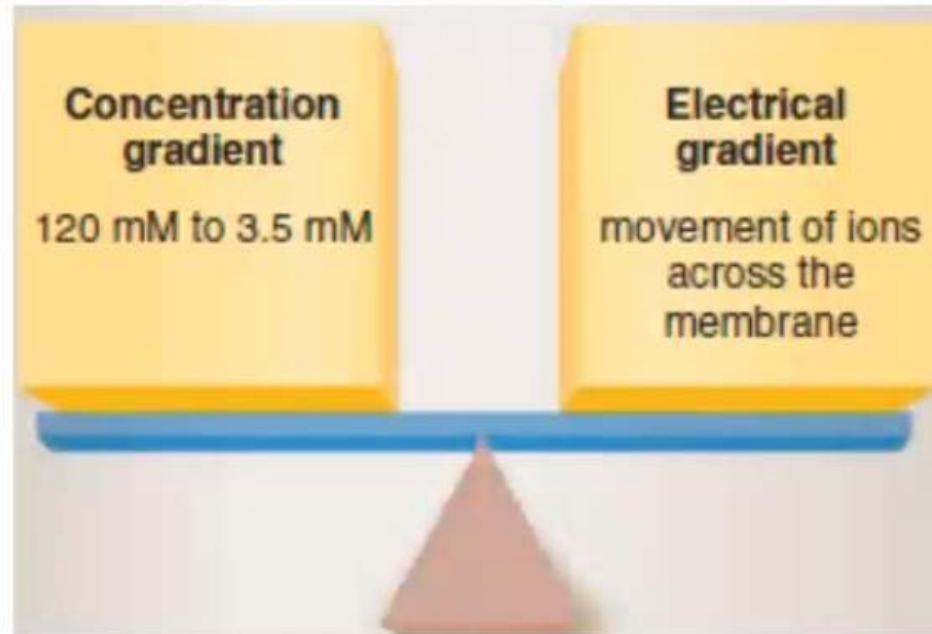
- 3** Equilibrium potential or electrochemical equilibrium is achieved when there is a balance between the concentration gradient and the electrical gradient.

Figure from Illustrated Reviews of Neuroscience by Claudia Krebs

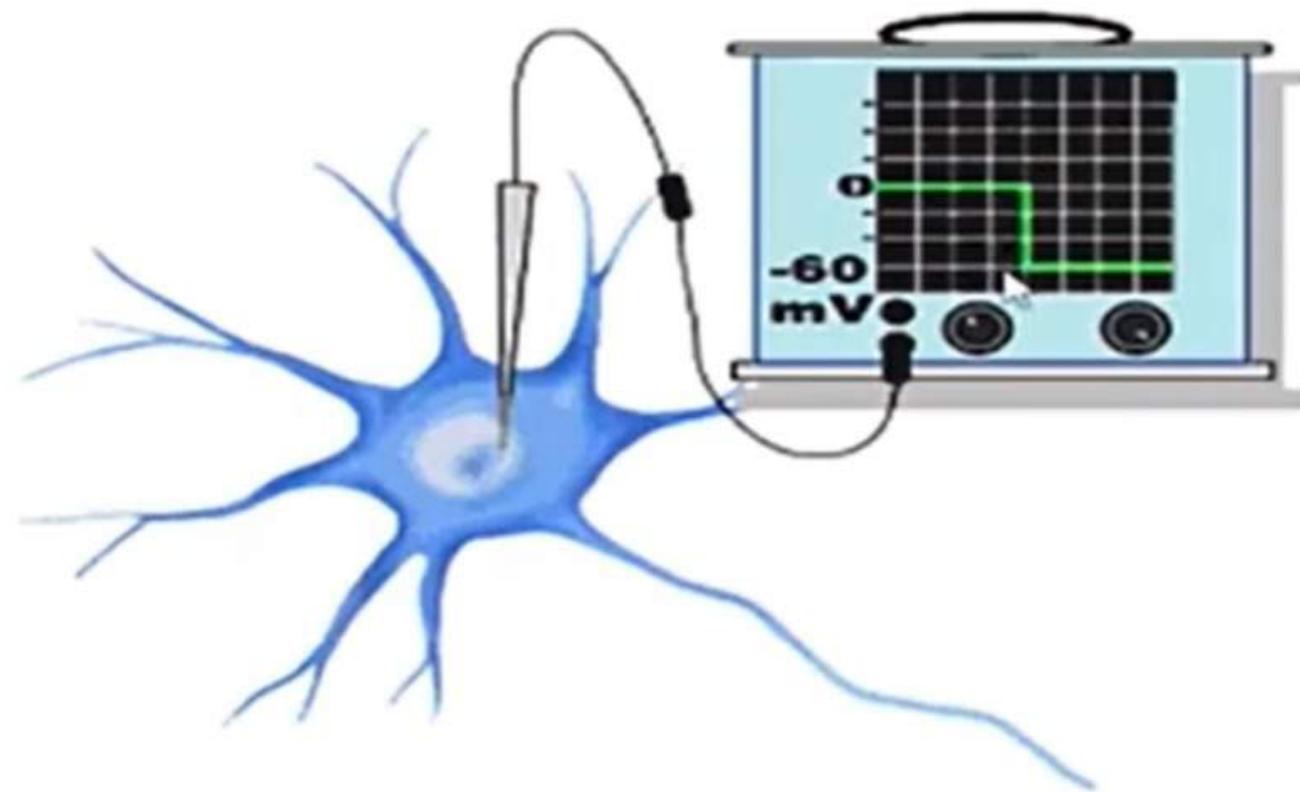
Recap-What concept is this



Recap-What concept is this



Recap-What concept is this



TODAY



1. Membrane Potential

2.Graded Potential

3.Action Potential

6th Slide is Missing

Membrane Proteins as Ion Pumps

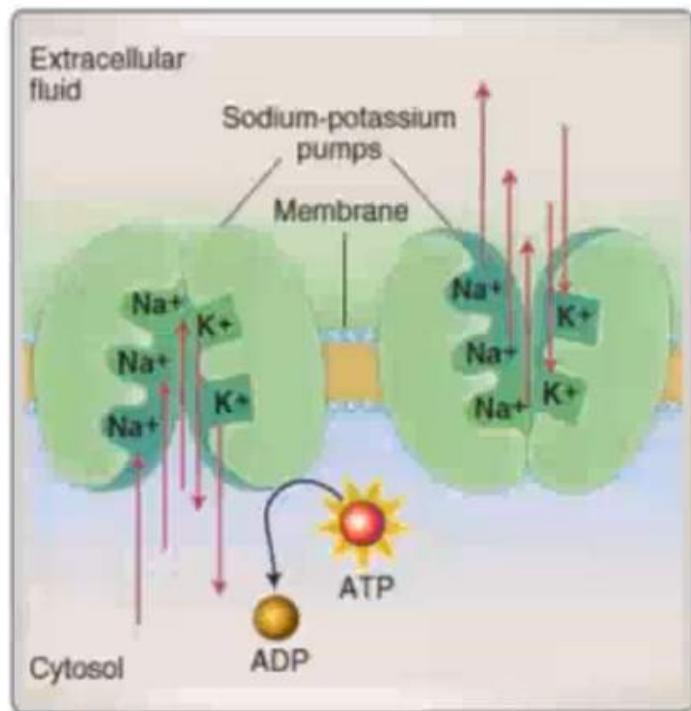


Figure 1.11

Ion pumps. ATP = adenosine triphosphate; ADP = adenosine diphosphate.

Na⁺/K⁺ ATPase,

Figure from Neuroscience
by Claudia Krebs

The different intracellular and extracellular ion concentrations are maintained by membrane proteins that act as **ion pumps**. The most prominent of these ion pumps is the **Na⁺/K⁺ ATPase**, which pumps Na⁺ (sodium) out of the cell in exchange for K⁺. This activity of the Na⁺/K⁺ exchanger is shown in Figure 1.11. As the name implies, these ion pumps depend on energy in the form of adenosine triphosphate (ATP) to function. The pump can only function in the presence of ATP, which is hydrolyzed to adenosine diphosphate (ADP) in order to release energy.¹

Membrane Proteins as Ion Pumps to
maintain intracellular and extracellular
concentrations

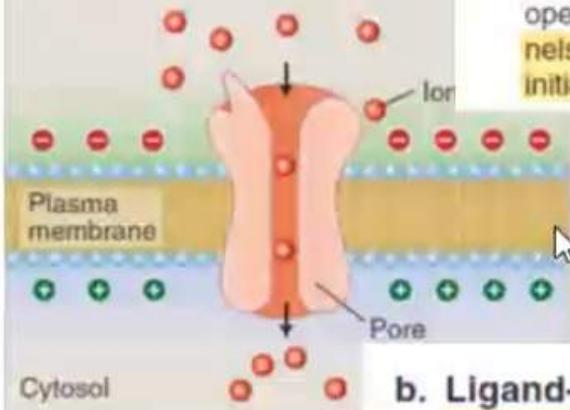
Membrane Proteins as Ion Pumps

Ion channels are membrane proteins that allow ions to pass through them, which causes current flow. Ion channels are selective: The size of the **channel pore** and the amino acids in the pore will regulate which ion can pass through. The opening or closing of ion channels is regulated by different mechanisms as detailed in Figure 1.12.



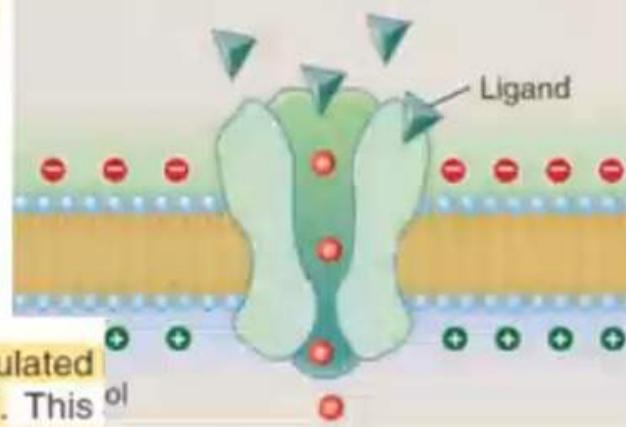
Ion Channels

A Voltage-gated channel



a. **Voltage-gated ion channels:** These channels are regulated by the membrane potential. A change in membrane potential opens the channel pore. The most prominent of these channels is the voltage-gated Na^+ channel. Its opening underlies the initiation of an action potential (see Figure 1.12A).

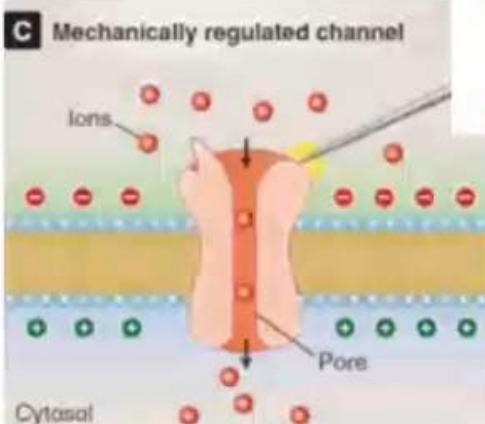
B Ligand-gated receptor



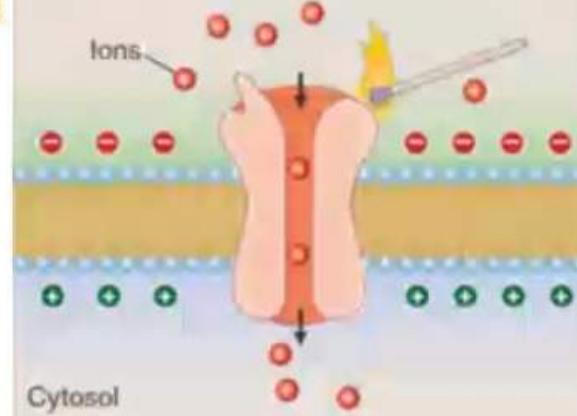
b. **Ligand-gated ion channels:** These channels are regulated by a specific molecule that binds to the ion channel. This opens the pore, and ions can pass through. Postsynaptic neurotransmitter receptors are ligand-gated ion channels (see

c. **Mechanically gated ion channels:** The pore in these channels is mechanically opened. Touch receptors in the skin and receptor cells in the inner ear are examples of mechanically gated ion channels. These channels open through the mechanical deflection that pries or pulls the channel open

C Mechanically regulated channel



D Thermally gated channel



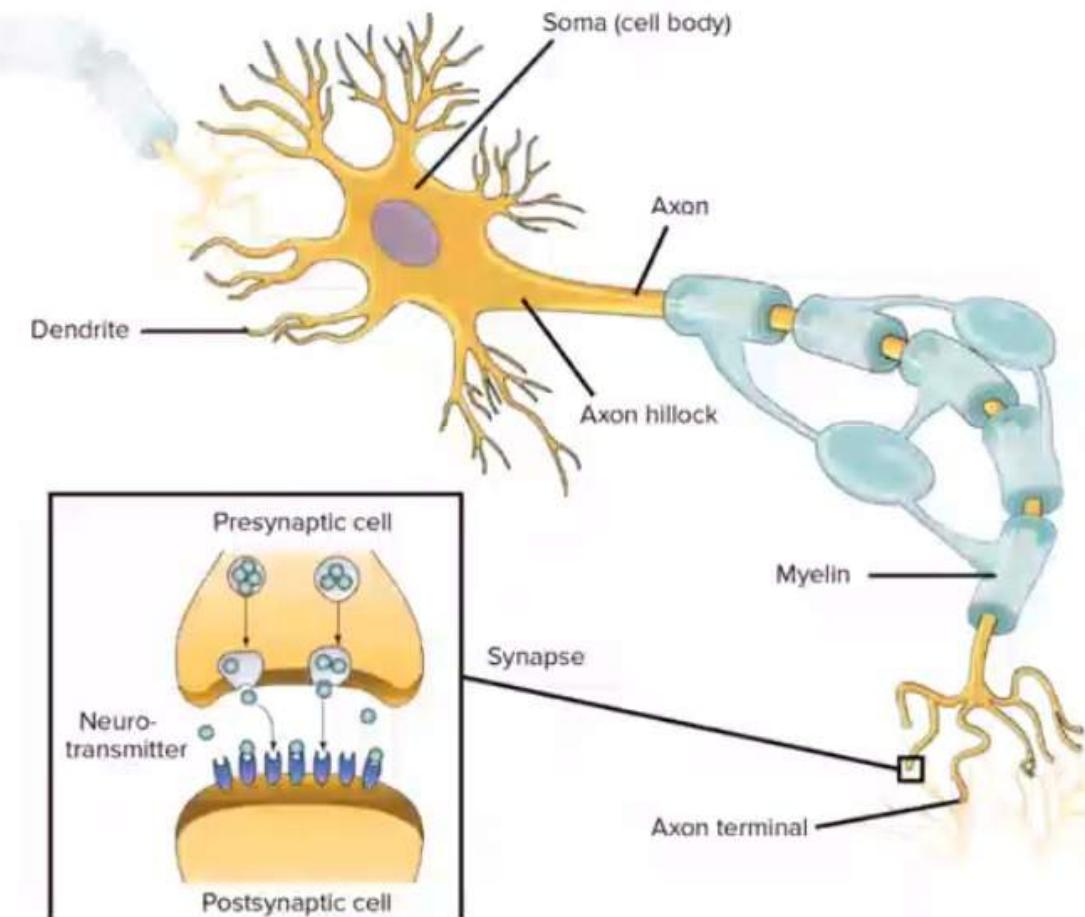
Thermally gated ion channels: These channels are regulated by temperature. The channel protein acts as a thermometer, and a change in temperature opens the channel pore



Concept of Neurotransmitters/Synapse ?

Neurotransmitters

- Chemicals known as neurotransmitters (glutamate, dopamine) are stored in membrane-bound vesicles at the axon terminal of neurons.
- Get released when Ca^{2+} enters the axon terminal and act by binding to receptors on the membrane of the postsynaptic cell.
- They are “**Excitatory**” firing a target neuron (Glutamate) “**Inhibitory**” making a target neuron less likely to fire (GABA).

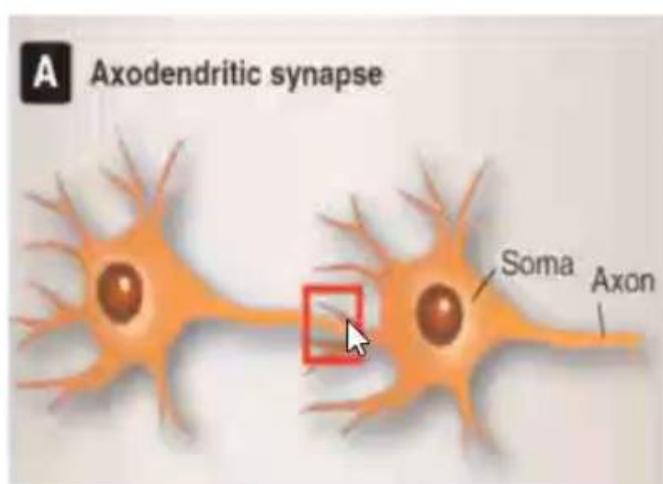


Neurotransmitters/Synapse

Types of Synapse?

Types of Synapse

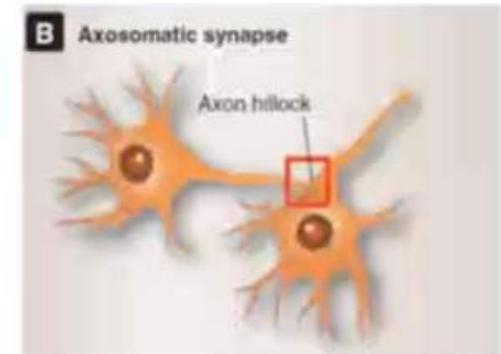
A Axodendritic synapse



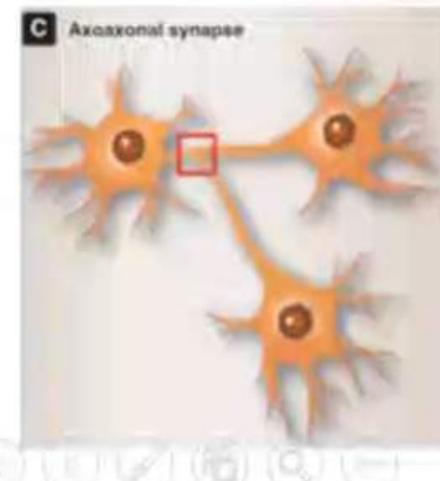
a. **Axodendritic synapses:** The most common synaptic contacts in the CNS are between an axon and a dendrite called **axodendritic synapses**. The dendritic tree of any given multipolar neuron will receive thousands of axodendritic synaptic inputs, which will cause this neuron to reach threshold (see below) and to generate an electrical signal, or **action potential**. The architecture of the dendritic tree is a key factor in calculating the convergence of electrical signals in time and in space (called **temporospatial summation**, see below).

b. **Axosomatic synapses:** An axon can also contact another neuron directly on the cell soma, which is called an **axosomatic synapse**. This type of synapse is much less common in the CNS and is a powerful signal much nearer to the axon hillock where a new action potential may originate.

B Axosomatic synapse



C Axoaxonal synapse

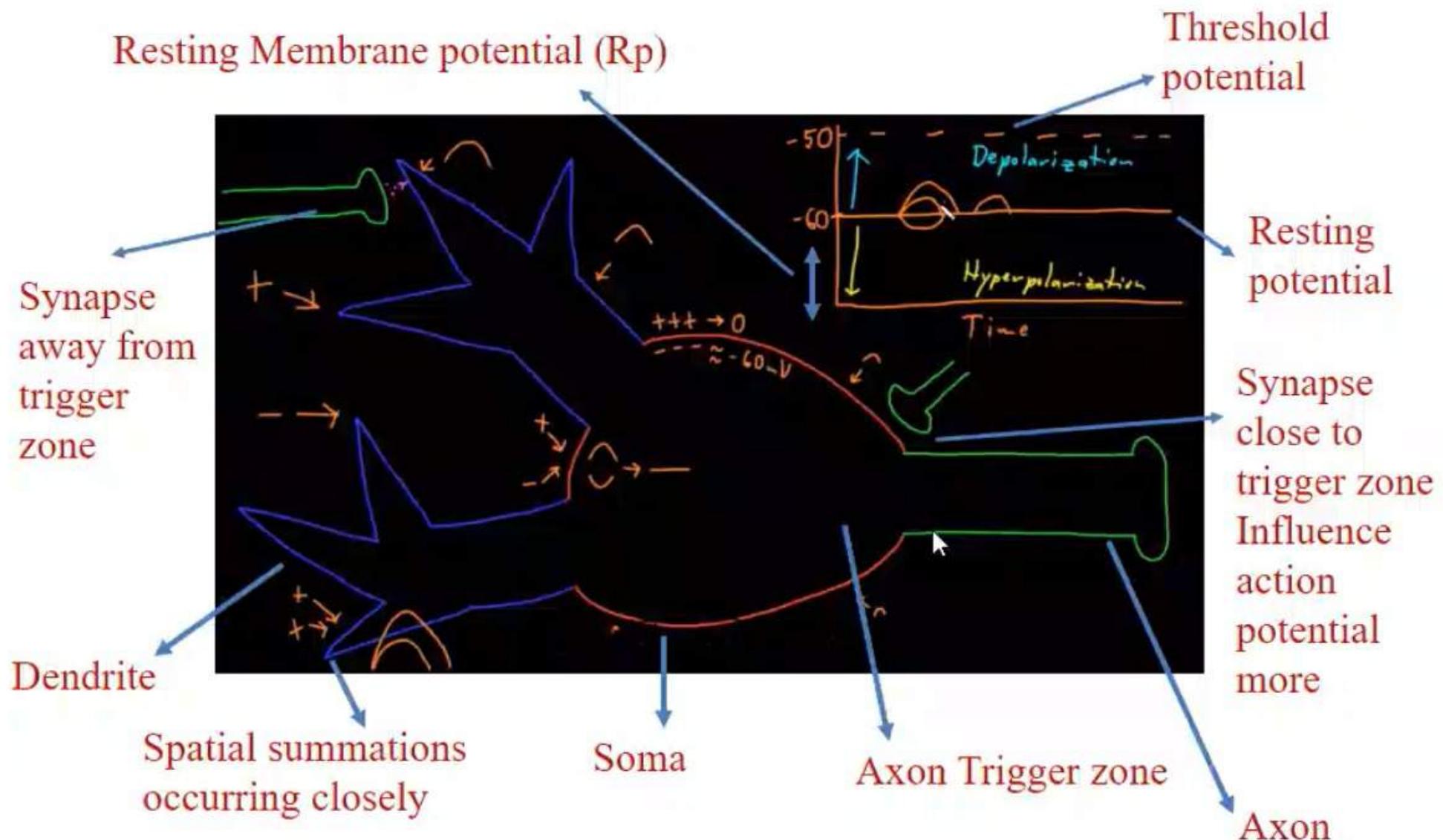


Axoaxonic synapses: When an axon contacts another axon, it is called an **axoaxonic synapse**. These synapses are often on or near the axon hillock where they can cause very powerful effects, potentially producing an action potential or inhibiting an action potential that would have otherwise been fired.



What is Graded Potentials??

Graded Potentials (Quick Look)



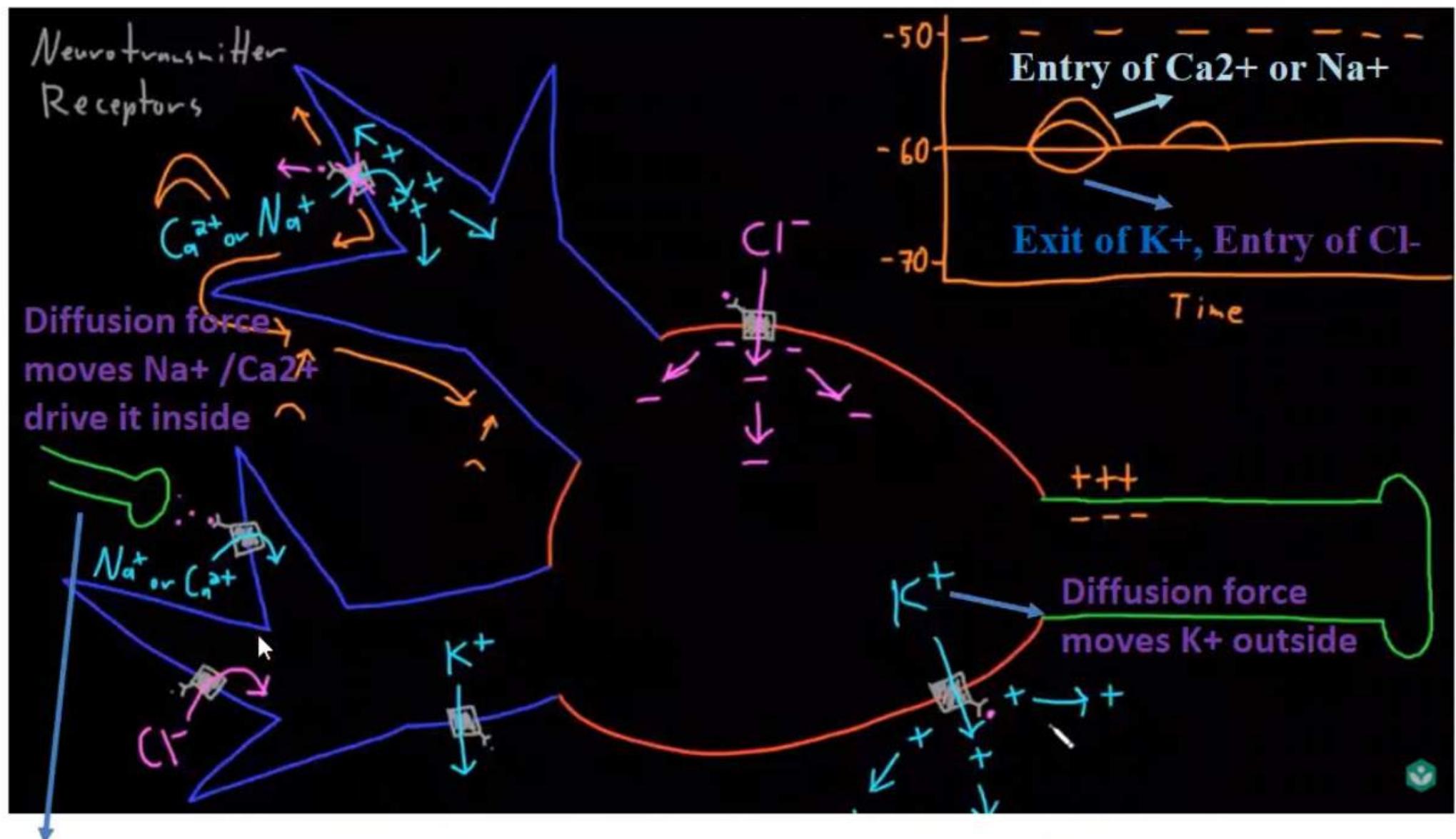
Modified Figure from Khan Academy

Graded Potentials

- *Transient membrane potential* changes occur in resting potential of neurons and are called *Graded potentials*.
- They occur in the *dendrites* and *soma* of the neuron.
- Excitatory input *depolarizes* while inhibitory input *hyperpolarizes* membrane potential.
→
- Size and duration of graded potentials is determined by size/duration of inputs (Excitatory and inhibitory).
- Graded potentials decay with time and distance.
- Graded potentials do not pass into the *axons of the neurons*.

Ion Flow during Graded potentials

Graded Potentials (Flow of Ions)



Ligand gated channel opens (when neurotransmitter binds to receptor)

Modified Figure from Khan Academy

Generation of Action Potential

Study how 100 billion Neurons are firing of nearly 50 action potentials per second thereby controlling what we do, how we think, move muscles, listen to lecture

Light Stimulus

PLACE CURSOR OVER COLOR CIRCLE

Dim



Bright

1 sec

Nerve Activity



Action Potential

Hartline HK.
Intensity and
duration in the
excitation of single
photoreceptor
units. *J Cell Comp
Physiol.* 5:229, 1934.

(140 years after
Galvani)

Light of varying
intensities

Light Stimulus

PLACE CURSOR OVER COLOR CIRCLE

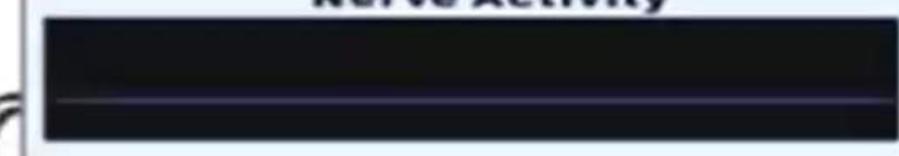
Dim



Bright

1 sec

Nerve Activity



Light Stimulus

PLACE CURSOR OVER COLOR CIRCLE

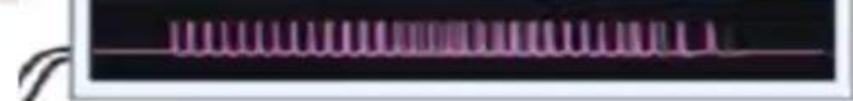
Dim



Bright

1 sec

Nerve Activity



Electrodes



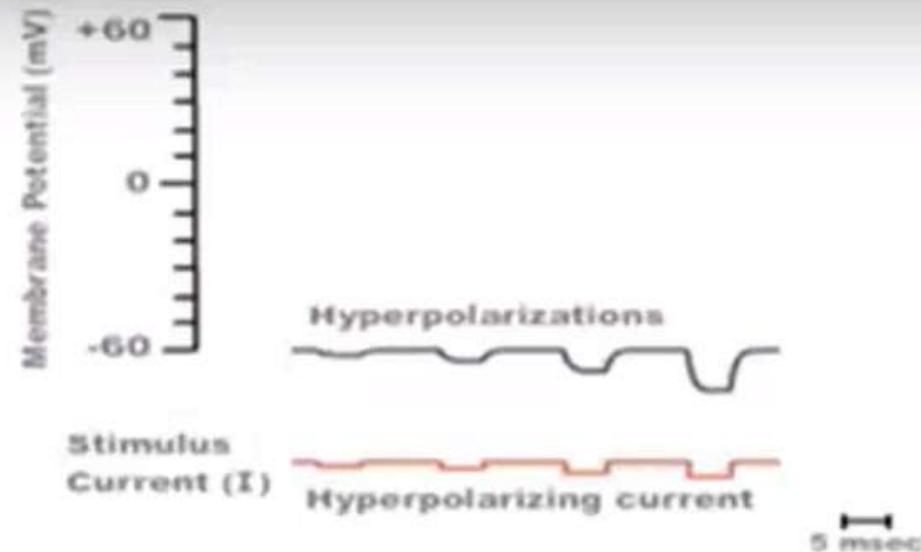
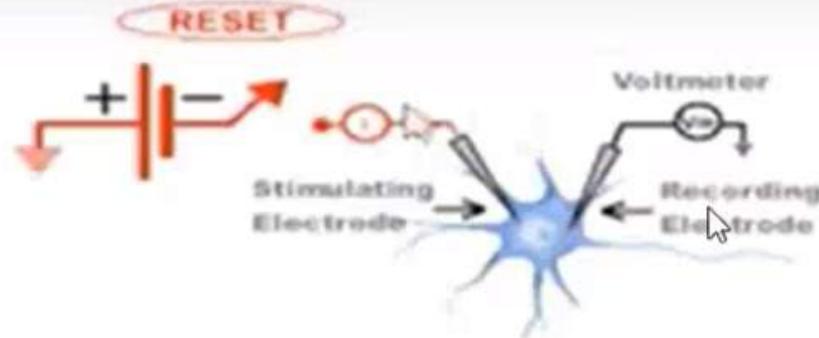
Optic Nerve



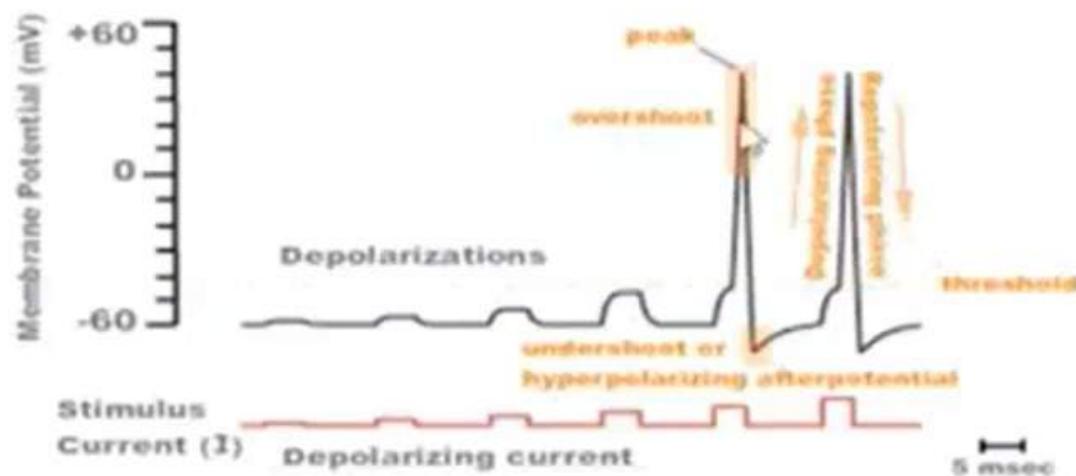
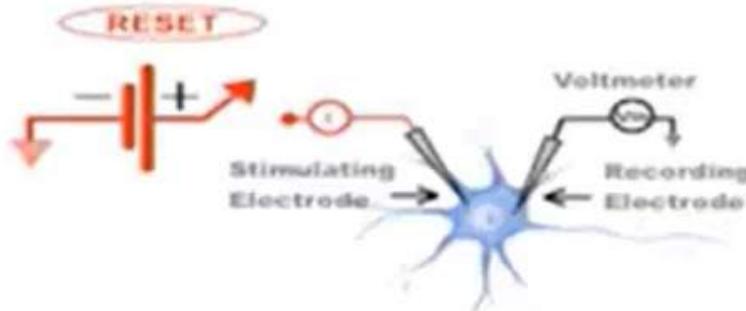
Squid fish

Action Potential

A. Hyperpolarizations



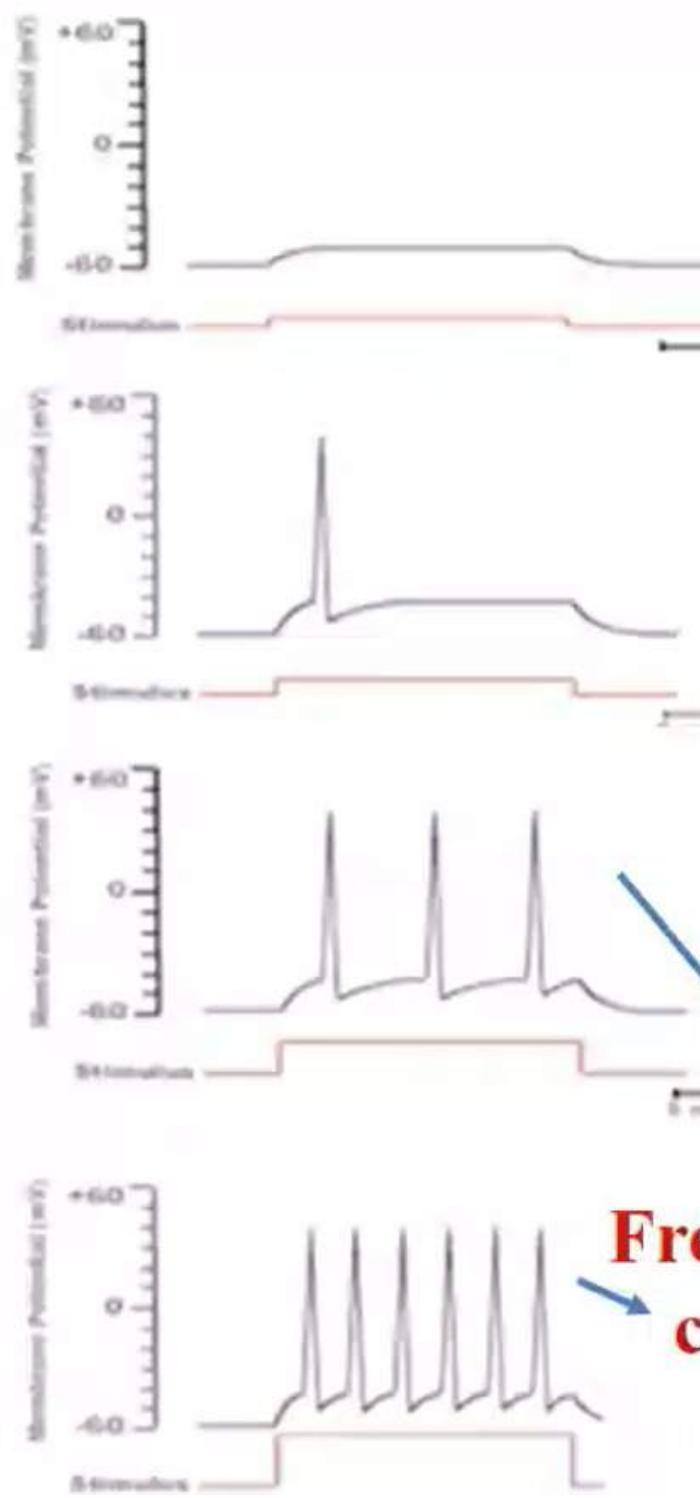
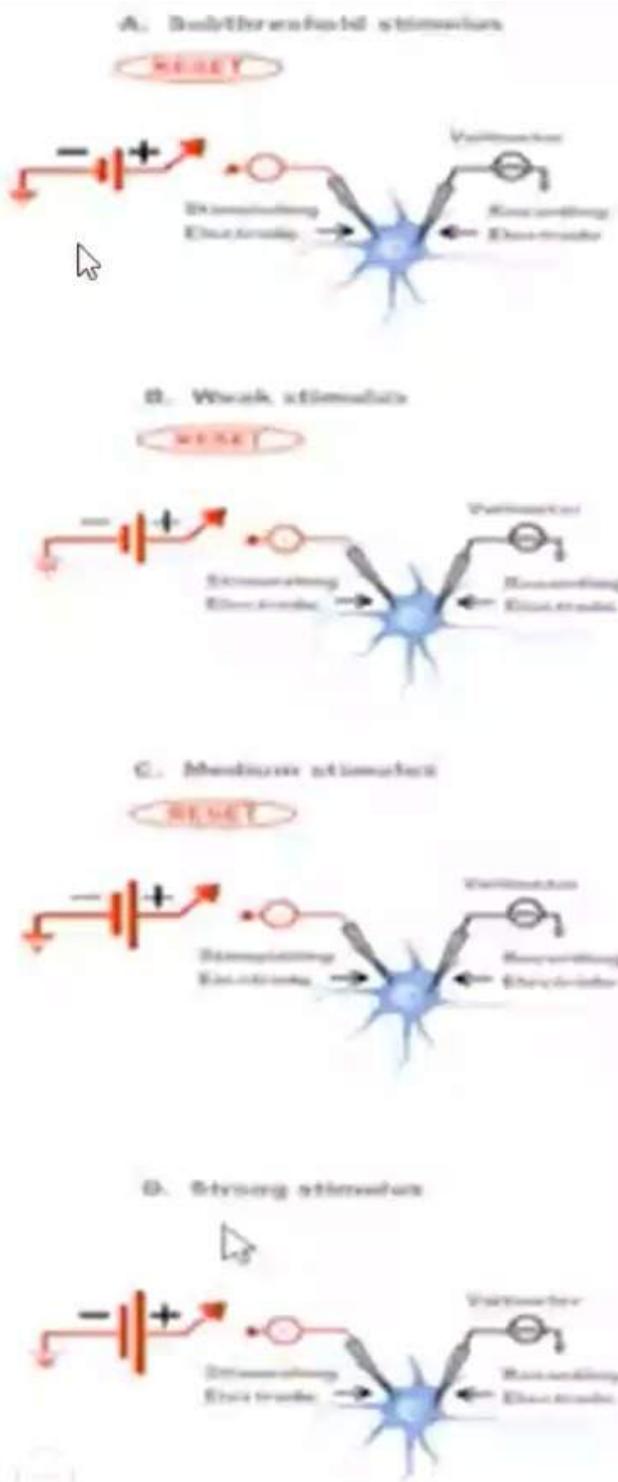
B. Depolarizations and the Action Potential



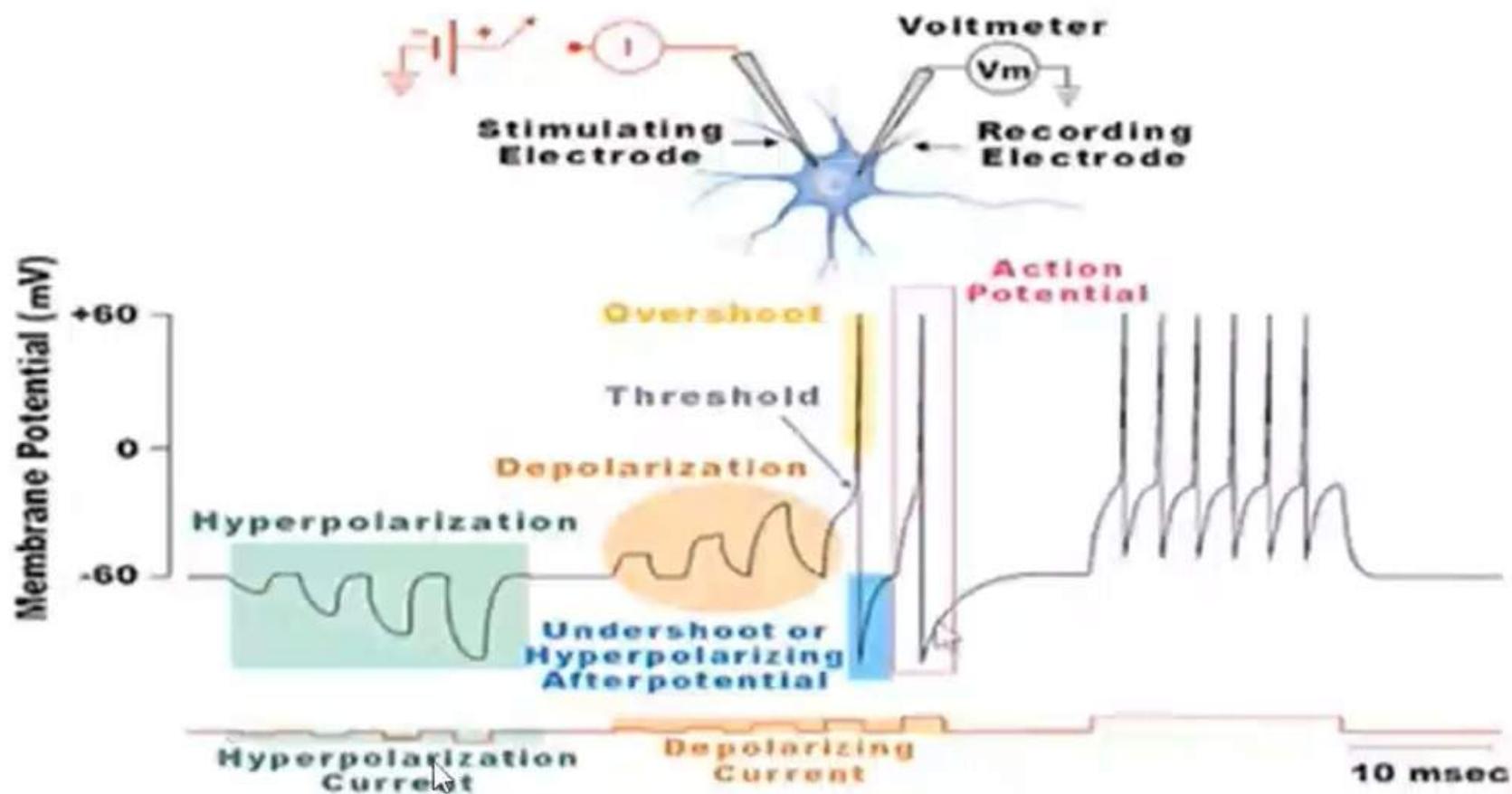
Action Potential (Electrical Viewpoint)



Battery power

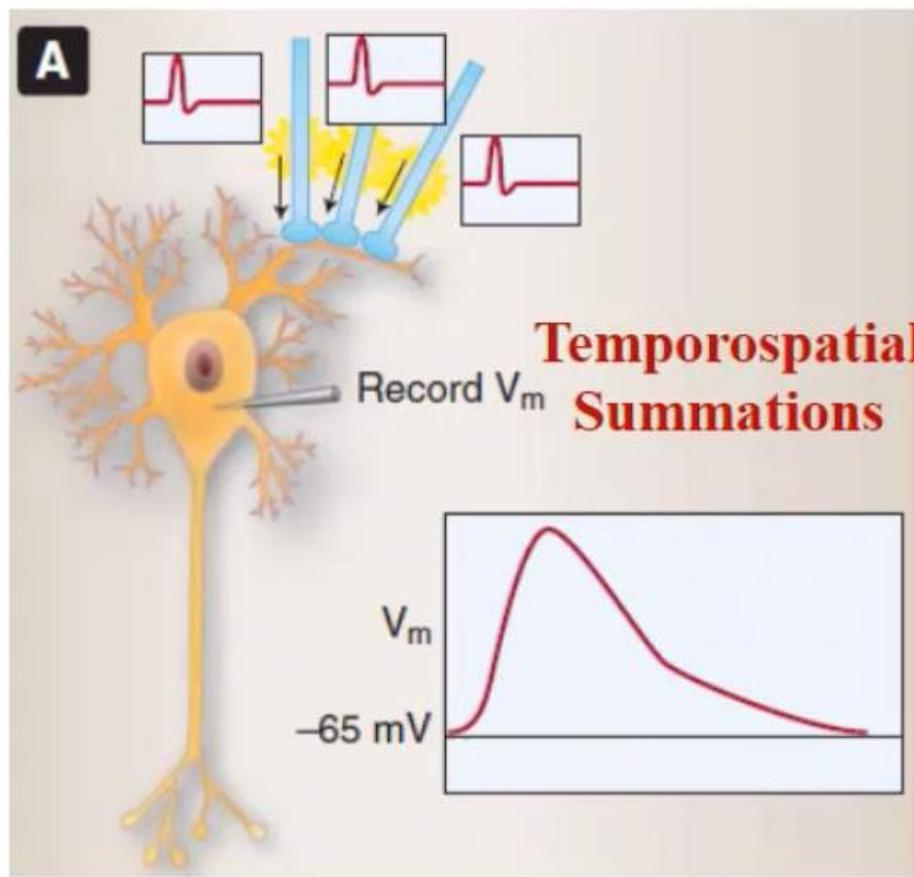


**Frequency
coding**

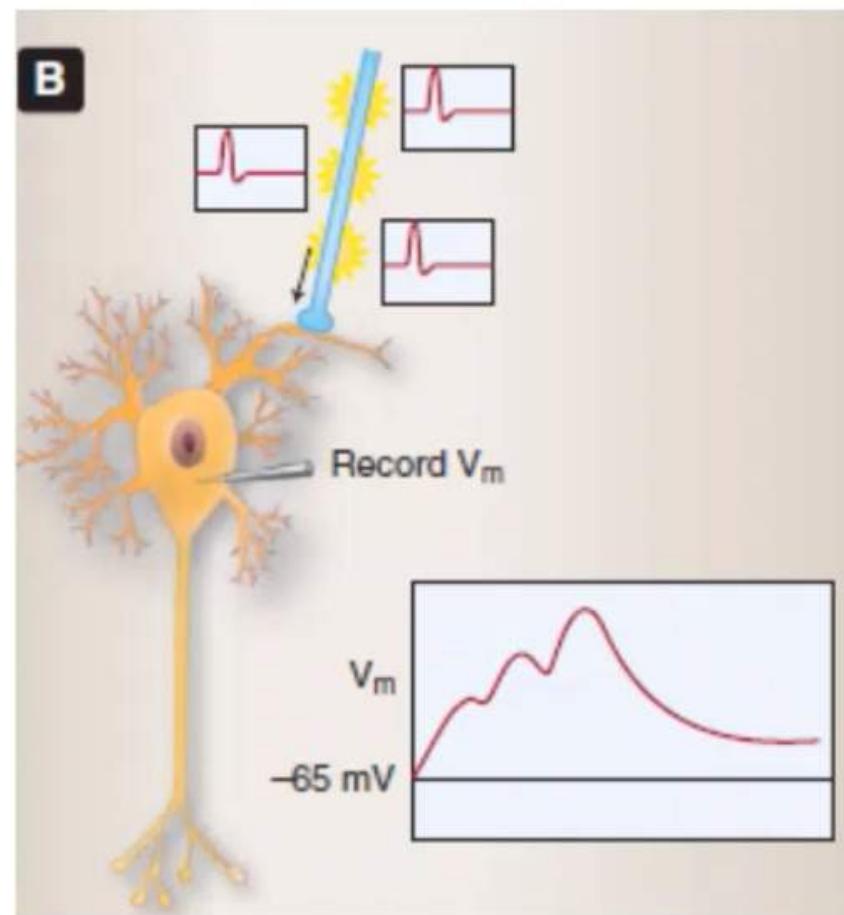


**Frequency
coding**

Summation of Graded Potential for AP



Temporospatial
Summations



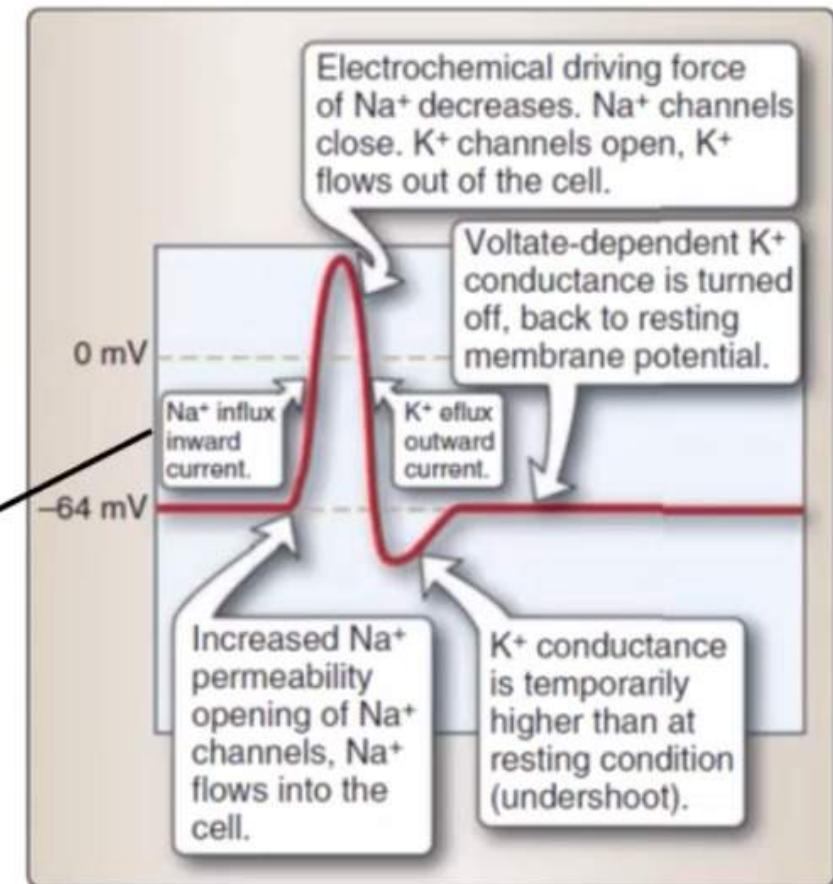
The synapses receiving input must be close together (Figure 1.22A) and receive input in the same timeframe (Figure 1.22B). This is termed **temporospatial summation**. Figure 1.22 shows how synaptically evoked potentials received at the same time and in the same area can bring the neuron close to threshold, which results in the generation of an AP.

Content from book: Neuroscience by Claudia Krebs

Generation of Action Potential (Ions Involved)

Action potentials (APs) are electrical impulses, or changes in membrane potential, that travel along the surface of a neuron. The underlying mechanism for APs is the change in membrane permeability for different ions, first Na^+ when initiating an AP and then K^+ in the recovery phase. APs are the means of communication between neurons.

Na^+ are voltage gated channels



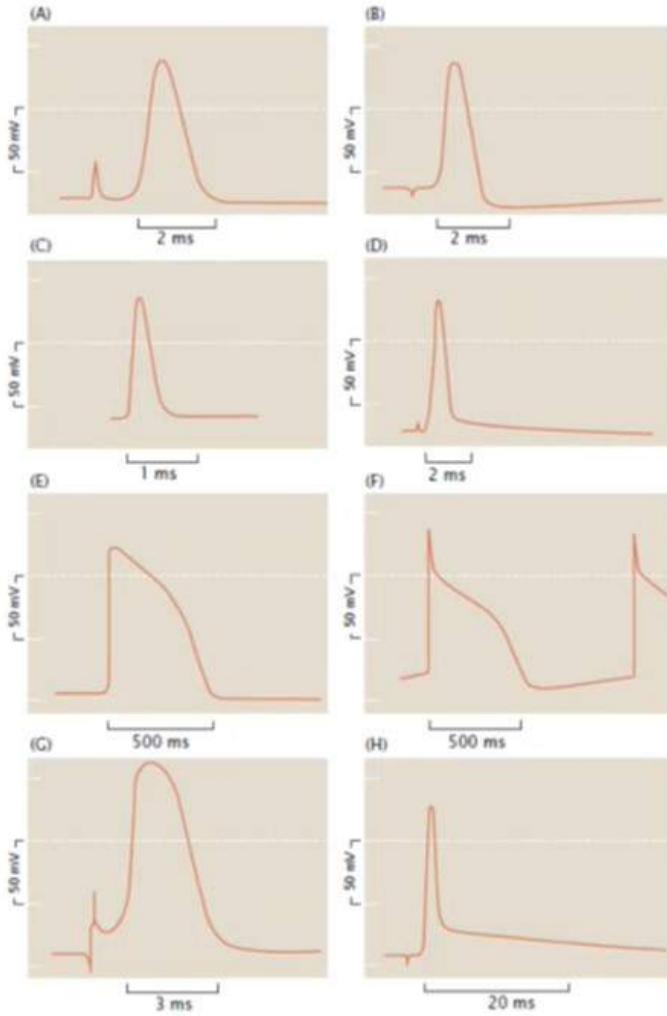
Content from book: Neuroscience by Claudia Krebs



MEMBRANE TRANSPORT_TODAYS LECTURE

- a. Neuron, Resting Potential, Nernst Equation Expression**
- b. Graded Potential/Action Potential**
- c. Ohms law, Electrical circuits, Hodgkin Huxley Model**

**Acknowledgment: Aggregated Lecture from lot of resources
(Textbooks/Coursera/Youtube lectures, Journal Papers)**



Action Potentials (Many Shapes)

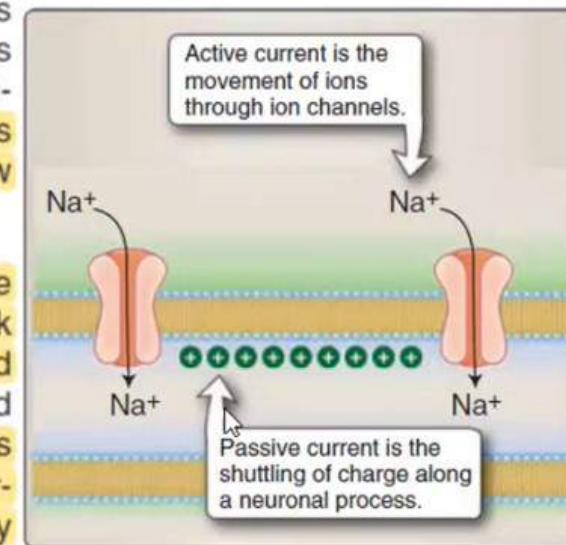
The many shapes of action potentials. This gallery shows examples of action potentials recorded from different cell types

Content from book: Physical Cell Biology

Propagation of AP (Active/Passive Currents)

2. **Propagation of action potentials:** The effective transmission of an electrical signal along an axon is limited by the fact that ions tend to leak across the membrane. The impulse will dissipate as charge is lost over the membrane. The AP has a way of circumventing the leakiness of the neuronal membrane. Electrical signals along an axon are propagated through both passive current flow and active current flow, as illustrated in Figure 1.14.

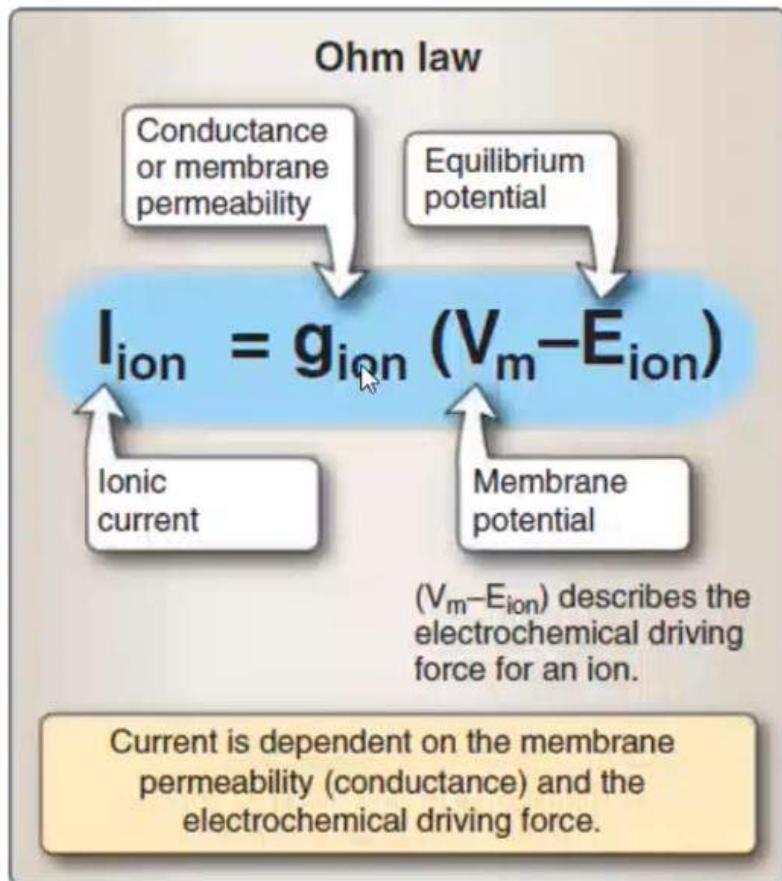
a. **Current:** Current is measured in amperes (A) and describes the movement of charge or movement of ions. The amount of work necessary to move that charge is described as the **voltage** and measured in volts (V). The difficulty of moving ions is referred to as **resistance** and measured in ohms (Ω). **Conductance** is the ease of moving ions and measured in siemens (S). The current of a specific ion depends on the membrane permeability (**conductance**) and the electrochemical driving force for that ion. This can be expressed by the **Ohm law**, which is summarized in Figure 1.15.



Passive current and active current.

- **Passive current:** Shuttling of charge like flow of electricity along a wire.
- **Active current:** Flow of ions (Na⁺) through ion channels





Ohms Law

Content from book: Neuroscience
by Claudia Krebs

Propagation of AP

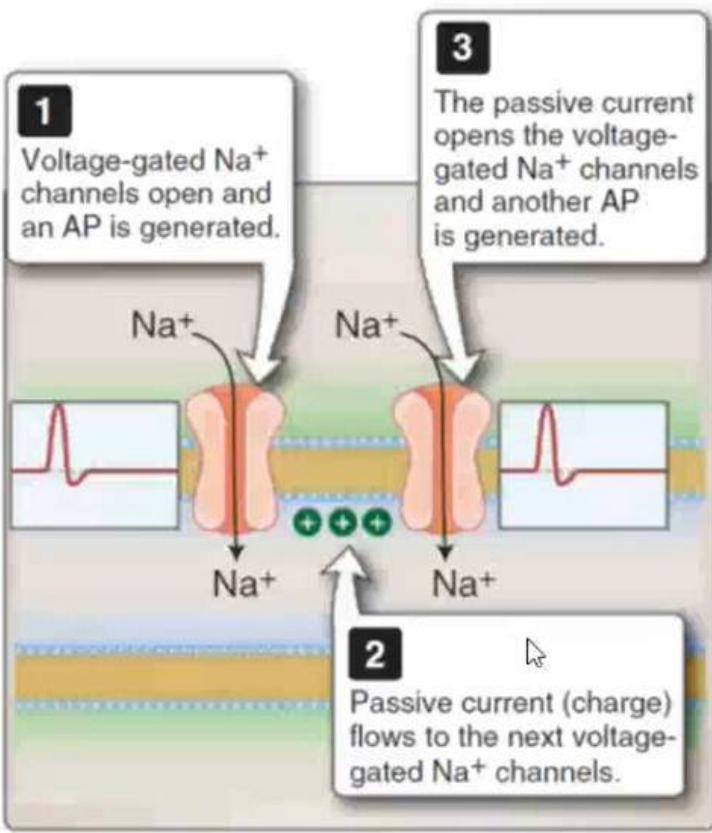


Figure 1.16

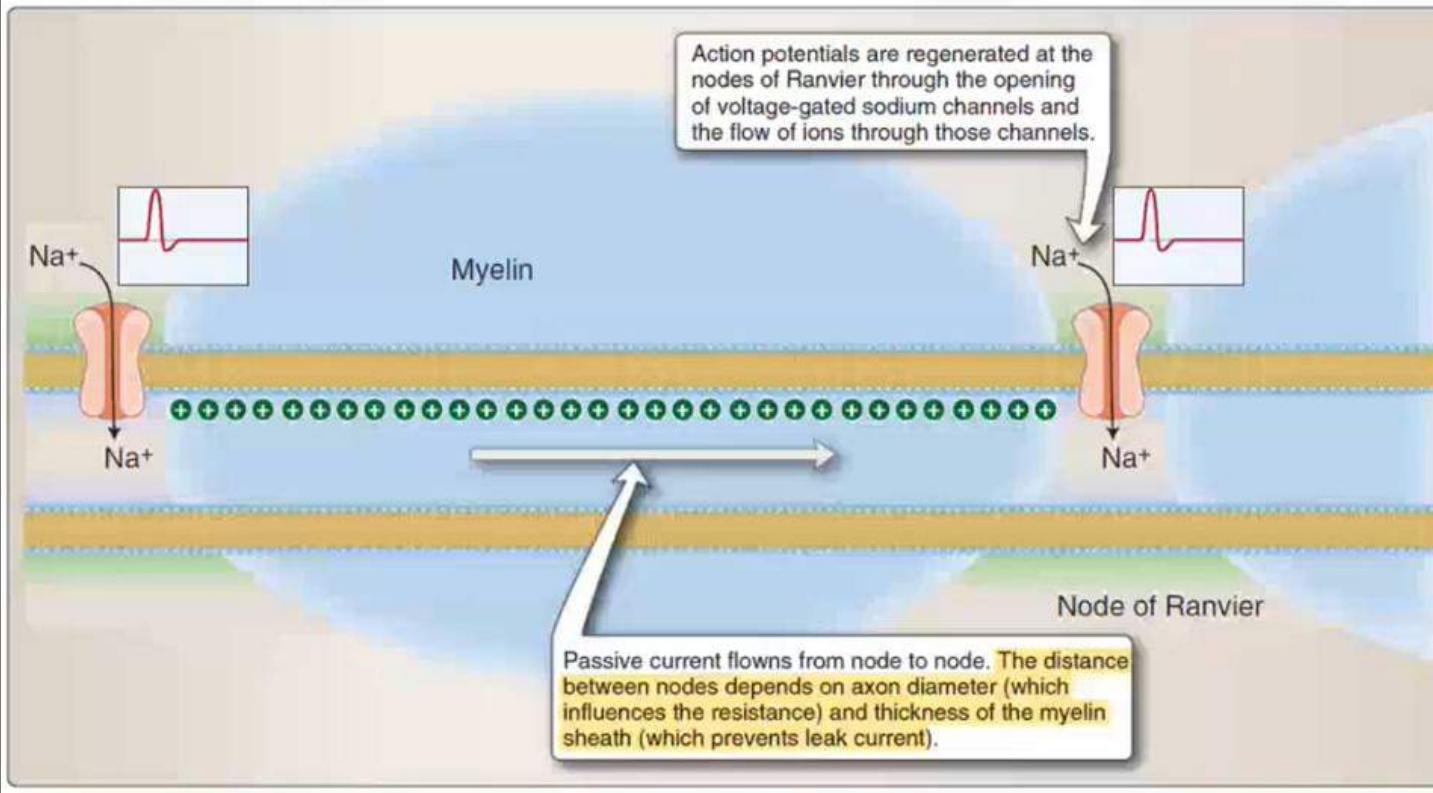
Continuous conduction.

Continuous conduction: In **unmyelinated** axons, passive current flows along the axon and continuously opens Na^+ channels (active current) that are inserted along the entire length of the axon. Continuous regeneration of APs along the entire length of axons is called **continuous conduction** and is illustrated in Figure 1.16.



Propagation of AP

Content from book:
Neuroscience by Claudia
Krebs

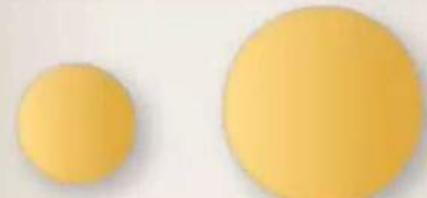


Saltatory conduction.

Saltatory conduction: In myelinated axons, Na⁺ channels are accumulated at the gaps in the myelin sheath (nodes of Ranvier). Passive current is shuttled along a long segment of the myelinated axon. At the node of Ranvier, the change in membrane potential causes the opening of Na⁺ channels and with that, the regeneration of the AP. The action potential seems to “jump” from node to node, which is called **saltatory conduction** and is illustrated in Figure 1.17.

1 Resistance

In larger axons, the resistance is lower, allowing for faster propagation of current.



2 Capacitance

In larger axons the membrane surface area is larger, increasing the capacitance, or amount of charge accumulated at the membrane.



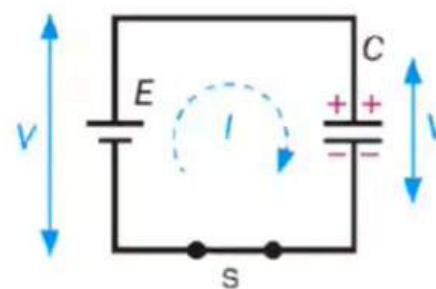
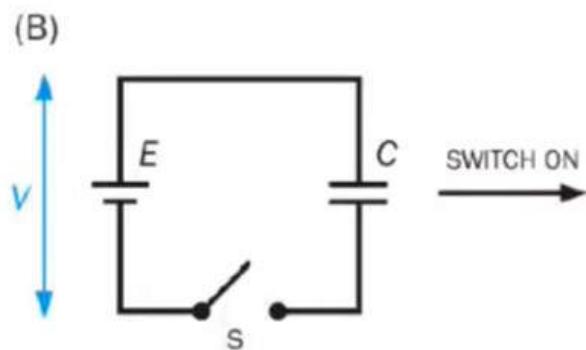
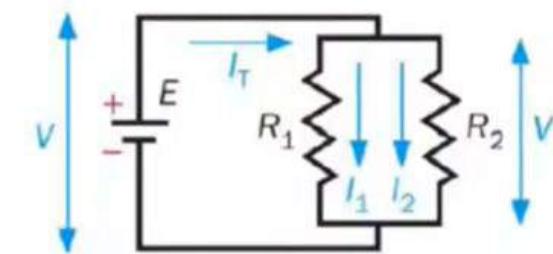
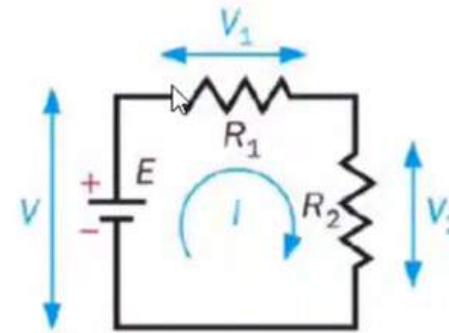
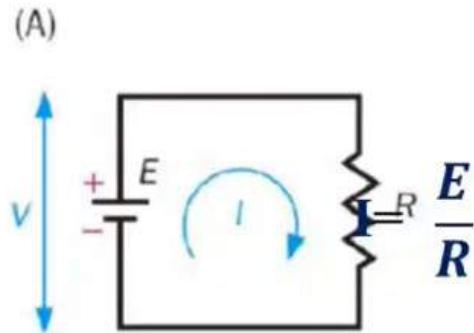
Resistance/Capacitance in Axons

Content from book: Neuroscience
by Claudia Krebs

Introduction to Hodgkin Huxley Model

**Extending Ohms law/Electrical Circuit Theory
(R,C and RC)**

Neuronal Plasma membrane or Biological membrane as Electrical circuits



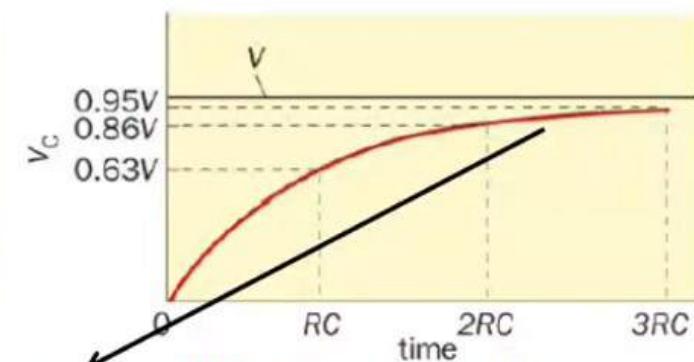
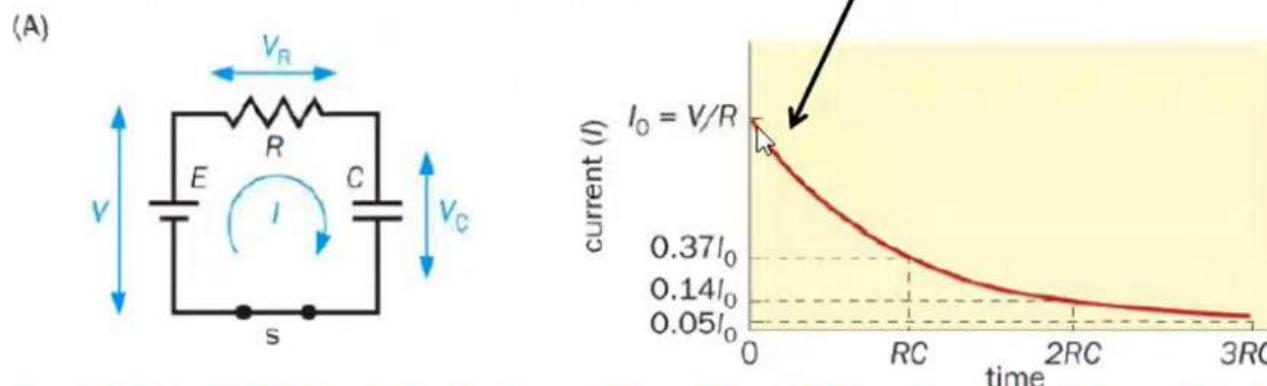
Luo L (2015) Principles
of Neurobiology.
Garland Science

Initially there is no charge across the Capacitor and it needs time (Current is biggest)

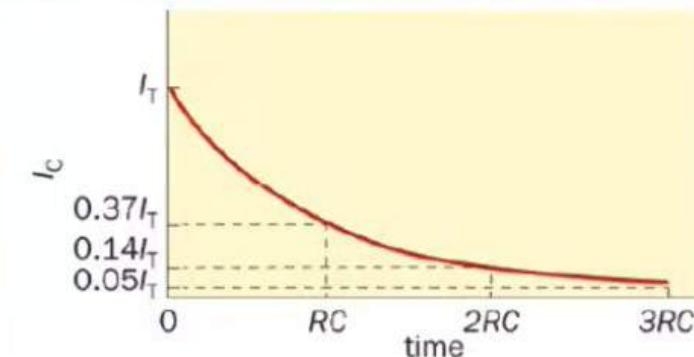
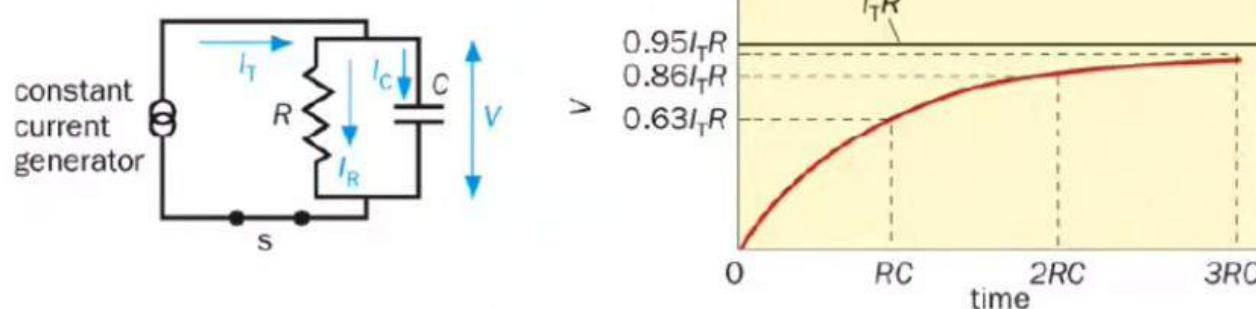
Over time capacitor gets charged and creates voltage same as battery E and current is zero

Resistor Capacitor InCombo

In RC circuits initially I is determined by R



In RC initially I is determined by R but with time C charges and determines the current I



Neuron membrane and RC circuit connection

J. Physiol. (1952) 117, 500–544

A QUANTITATIVE DESCRIPTION OF MEMBRANE
CURRENT AND ITS APPLICATION TO CONDUCTION
AND EXCITATION IN NERVE

BY A. L. HODGKIN AND A. F. HUXLEY

From the Physiological Laboratory, University of Cambridge

What is Hodgkin Huxley Model?
(23426 citations on Google scholar)
Nobel Prize in Physiology or Medicine, 1963

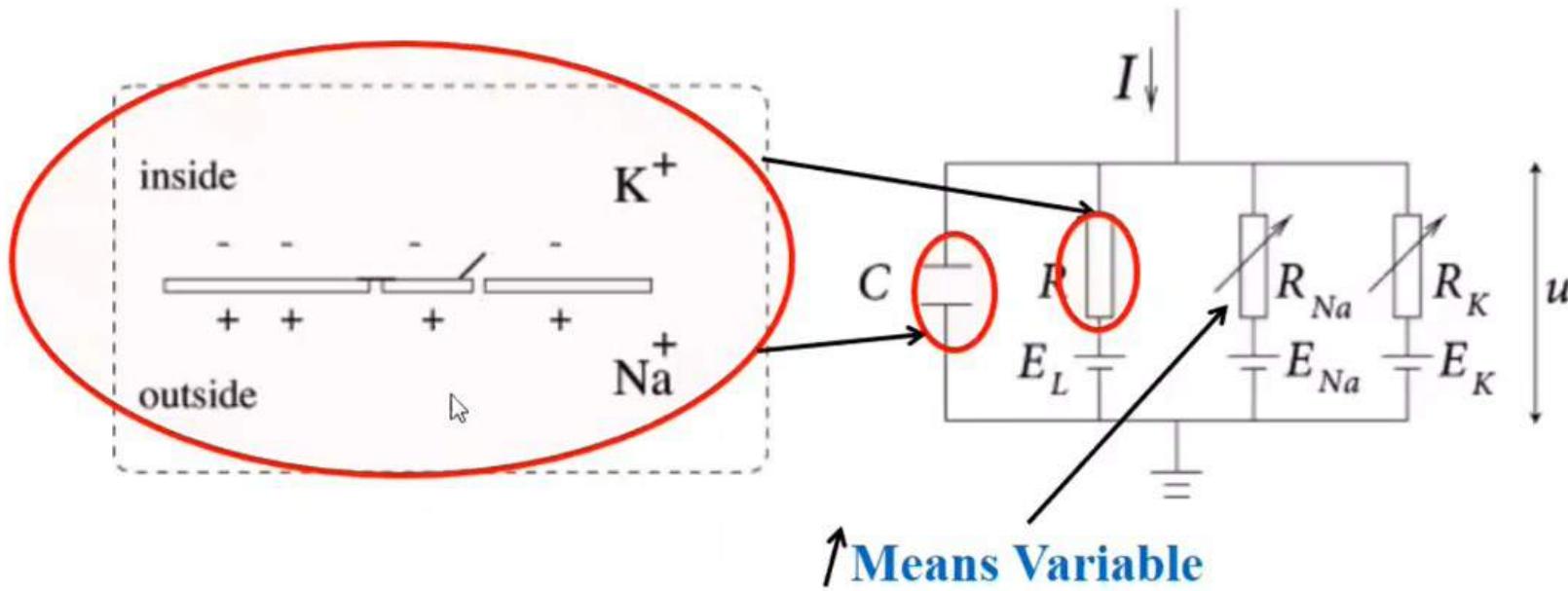


Electrical circuit models can be used to model ion flow across the neuronal membrane.



Lets look and Derive

Hodgkin Huxley (HH) Model



Semi permeable membrane as
RC circuit

Electrical circuit
representing ion flow



Hodgkin Huxley Model

- *Semi permeable membrane* acts as a capacitor
- Each channel type (Na^+ , K^+) is represented/characterized by a *resistor*.
- *Nernst potential* generated by differences in ion is represented by battery. E_L is leakage voltage for Cl^- and other ions
- Specific batteries since Nernst potential is different for each ion type.
- If input current $I(t)$ is injected into the neuron cell it charges the capacitor or leaks through the channels in cell membrane.

Hodgkin and Huxley, Journal of Physiology, 1952



Hodgkin Huxley (HH) Model

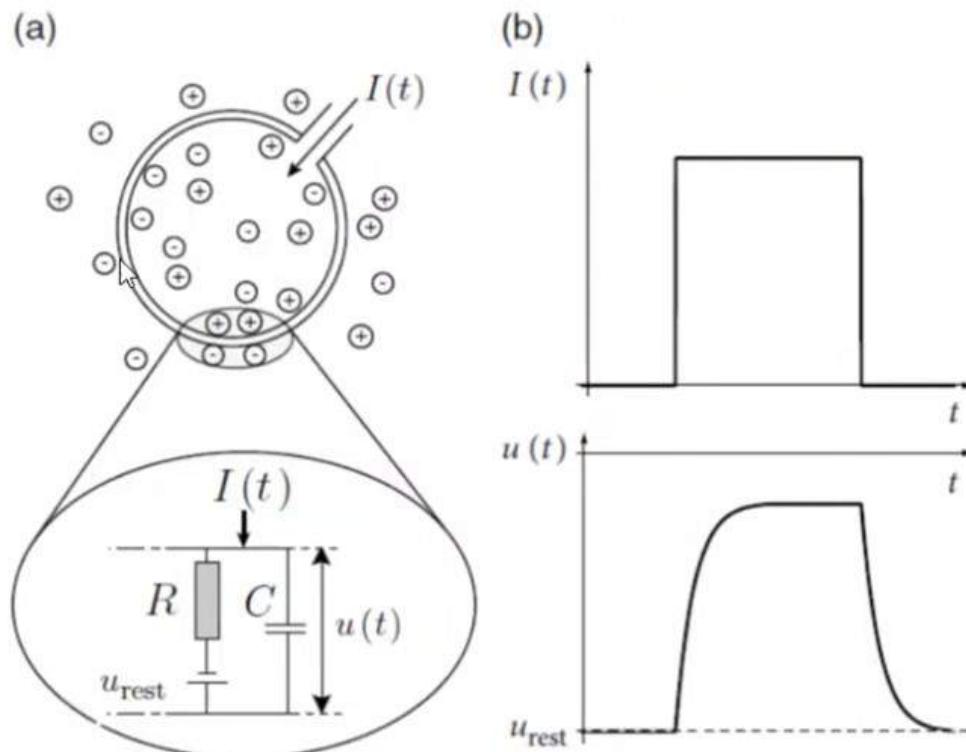


Fig. 1.6 Electrical properties of neurons: the passive membrane.
(a) A neuron, which is enclosed by the cell membrane (big circle), receives a (positive) input current $I(t)$ which increases the electrical charge inside the cell. The cell membrane acts like a capacitor in parallel with a resistor which is in line with a battery of potential u_{rest} (zoomed inset). (b) The cell membrane reacts to a step current (top) with a smooth voltage trace (bottom).

Electrical Circuit into Math equations

- Conservation of electric charge on membrane implies $I(t)$ is split as capacitive current and current which passes through ion channels (k in number).

$$I(t) = I_C(t) + \sum_k I_k(t)$$

$$C \frac{du}{dt} = - \sum_k I_k(t) + I(t)$$

- In biological terms u is the voltage across membrane and $\sum_k I_k$ is the sum of ionic currents which pass through the membrane

$C = \frac{qV}{u}$ = charge voltage

$qV = C u$

$\frac{dqV}{dt} = C \frac{du}{dt}$

$I_C = C \frac{du}{dt}$ (A)

Hodgkin and Huxley, Journal of Physiology, 1952



Electrical Circuit into Math equations

- Since u is the total voltage across the cell membrane and E_L is the voltage of battery, voltage of leak resistor is $u - E_L$
 $I_L = g_L (u - E_L)$ g_L : is conductance (inverse of resistance)

$$g_L = \frac{1}{R_L} \quad I_L = \frac{V}{R_L} = g_L(u - E_L)$$

- The mathematics of the other ion channels is analogous except that their conductance is voltage and time dependent.
- If all channels are open, they transmit currents with a maximum conductance.

Hodgkin and Huxley, Journal of Physiology, 1952

Electrical Circuit into Math equations

- $$\sum_k I_k = g_{\text{Na}} m^3 h (u - E_{\text{Na}}) + g_{\text{K}} n^4 (u - E_{\text{K}}) + g_L (u - E_L)$$
- Additional 'gating' variables m and h (Na^+ channels) and n (K^+ channels) modeled the probability that a channel is open at a given moment in time.
- The effective conductance of sodium channels is modeled as $g_{\text{Na}} m^3 h$ where m describes channel opening and h its inactivation.
- Potassium channel conductance is modeled as $g_{\text{K}} n^4$ where n describes the activation of channel

Breakthrough of HH Model

- The breakthrough of Hodgkin and Huxley was that they succeeded to measure how the cell membrane voltage or current can be modeled and represented as electrical circuits



Hodgkin and Huxley, Journal of Physiology, 1952

Applications of Hodgkin Huxley Model

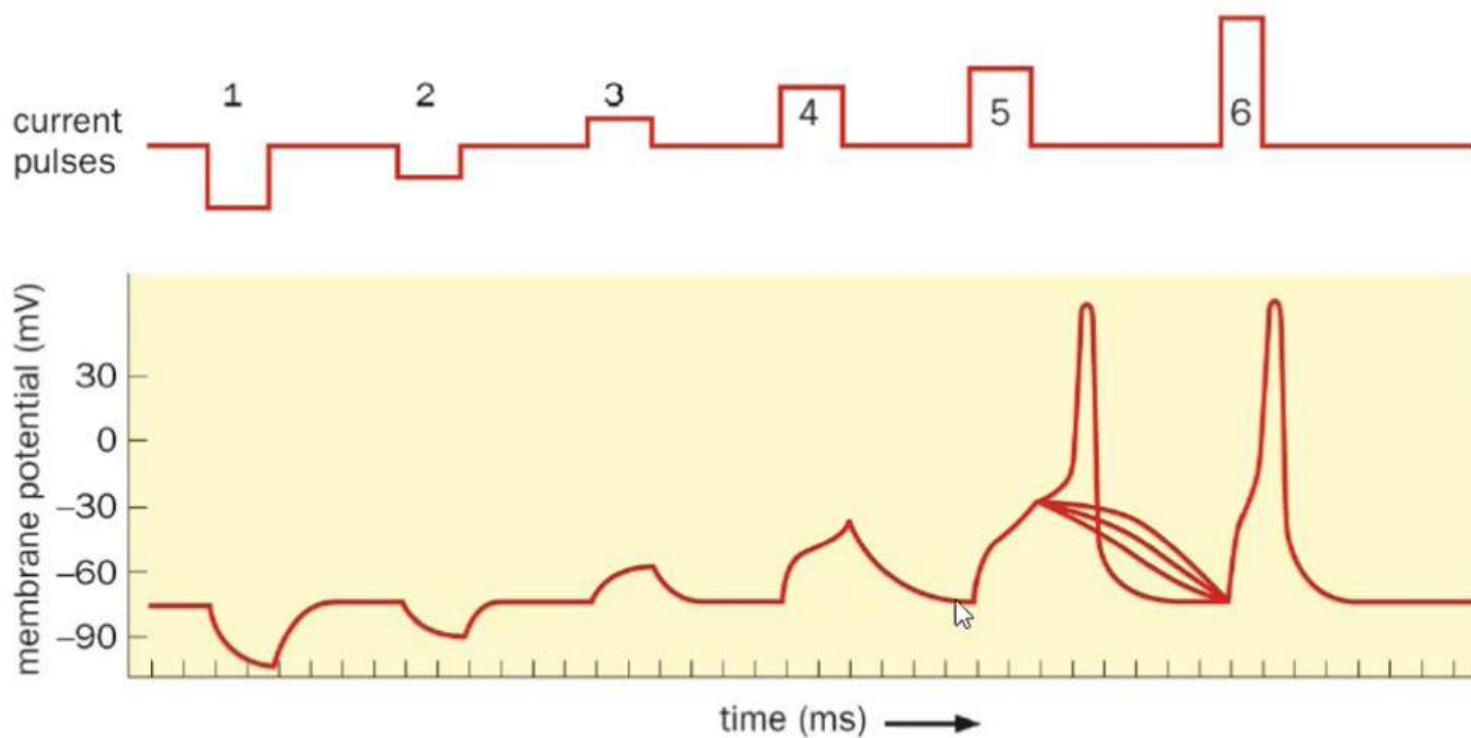
- Mathematical modeling can reveal mechanisms long before they can be observed directly.
- Framework for studying and analyzing ion channel kinetics.



Hodgkin and Huxley, Journal of Physiology, 1952



Neuron active property



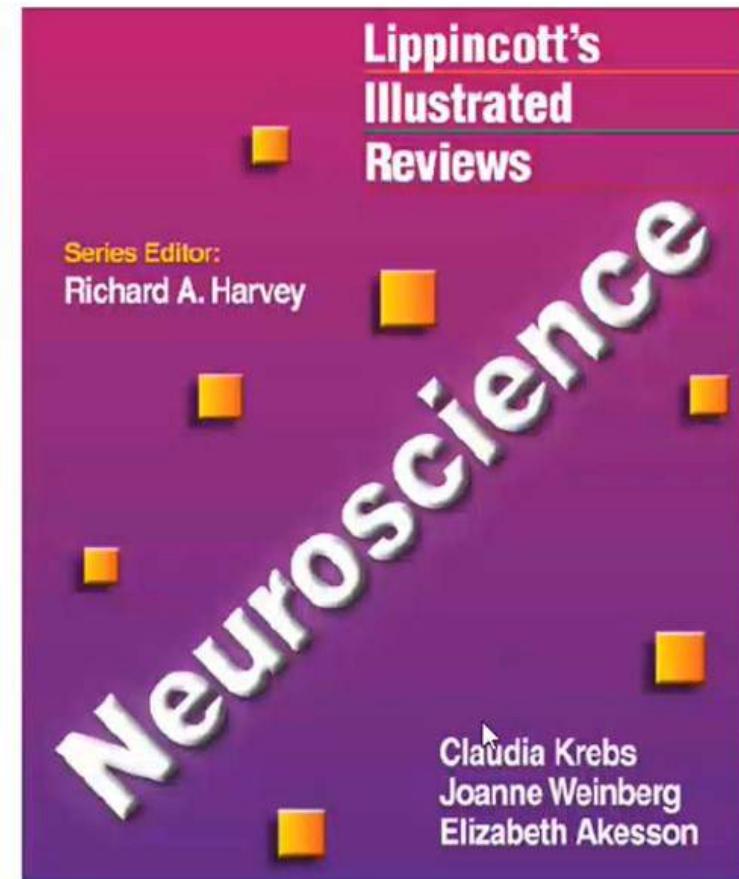
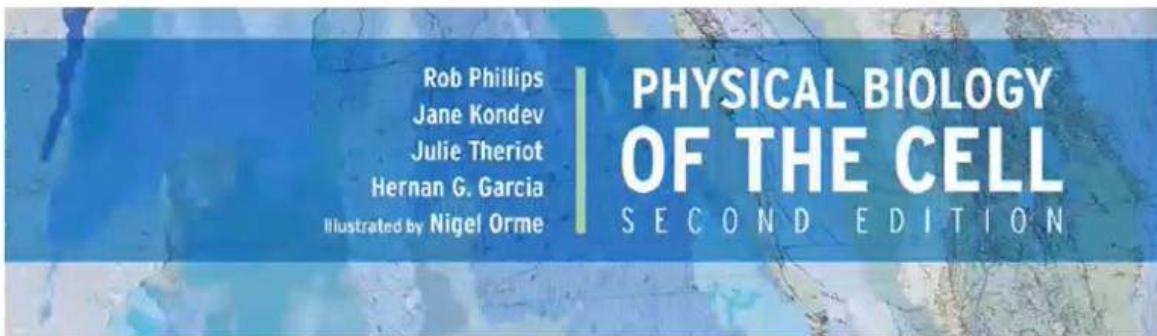
Katz (1966) Nerve muscle

Ion channels-related diseases

Neuronal disorders like Epilepsy, Alzheimer's disease, Parkinson's disease, Schizophrenia may result from dysfunction of voltage-gated sodium, potassium and calcium channels

Beata Dworakowska, Acta Biochim Pol. 2000

BT301 Lecture Sources





MODEL MEMBRANE -LIPOSOMES

- a. Lipids connection to Liposomes
- b. Introduction to Liposomes
- c. Biophysical properties of Liposomes
- d. Liposomes and skin: From Drug delivery to model membranes

Recorded with Hop Screen Recorder

Acknowledgment: Aggregated Lecture from lot of resources
(Textbook/Journal Papers/Youtube lectures)



Liposome: classification, preparation, and applications

Nanoscale
Horizons

REVIEW



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Cite this: *Nanoscale Horiz.*, 2021,

6(8), 1–18

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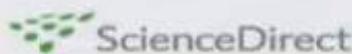
Targeted liposomal drug delivery: a nanoscience and biophysical perspective

Yibo Liu,^{a,b} Karla M. Castro Bravo^a and Juewen Liu^{a,c*}



EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 34 (2008) 303–322

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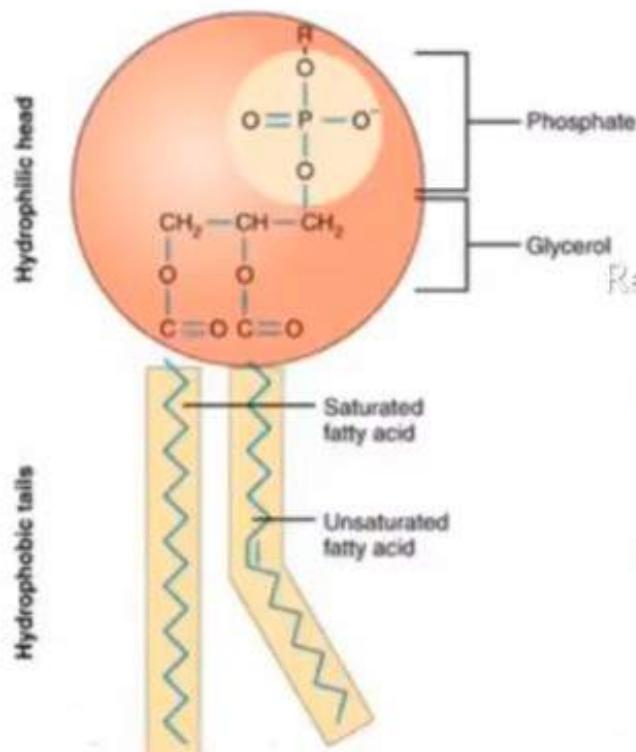


Review

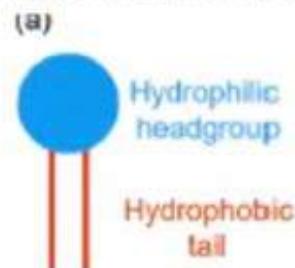
Liposomes and skin: From drug delivery to model membranes

Introduction to Lipids

A typical lipid molecule contains a hydrophilic headgroup and two hydrophobic tails (Fig. 1a). The charge of lipids and their chemical properties can be varied by changing the headgroup, while the hydrophobic tails mainly govern the packing in membranes.



Schematic illustration of (a) a lipid .



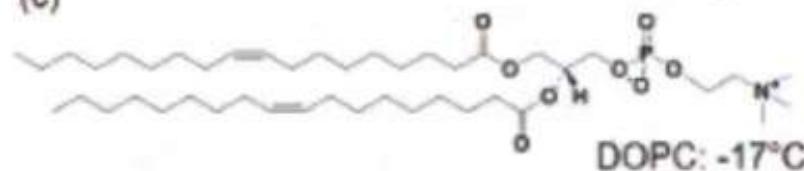
The tail structures can be changed rendering different phase transition temperatures (T_c). T_c is an important parameter that governs the fluidity of lipid bilayers. Above T_c , lipid tails have gauche conformation and can diffuse more freely, and the membrane exist in a liquid crystalline phase.²⁸ Below T_c , lipid tails are extended and diffuse slowly, and the membrane is in a gel-like state.

- *Phospholipids are a special group of lipids containing phosphate.*
- *Lipids in general are hydrophobic, also called non-polar. However, the phosphate group in phospholipids is hydrophilic, also called polar.*

Introduction to Lipids

A vast number of lipids are found in nature and more are available *via* chemical synthesis. A few commonly used lipids are listed in Fig. 1.

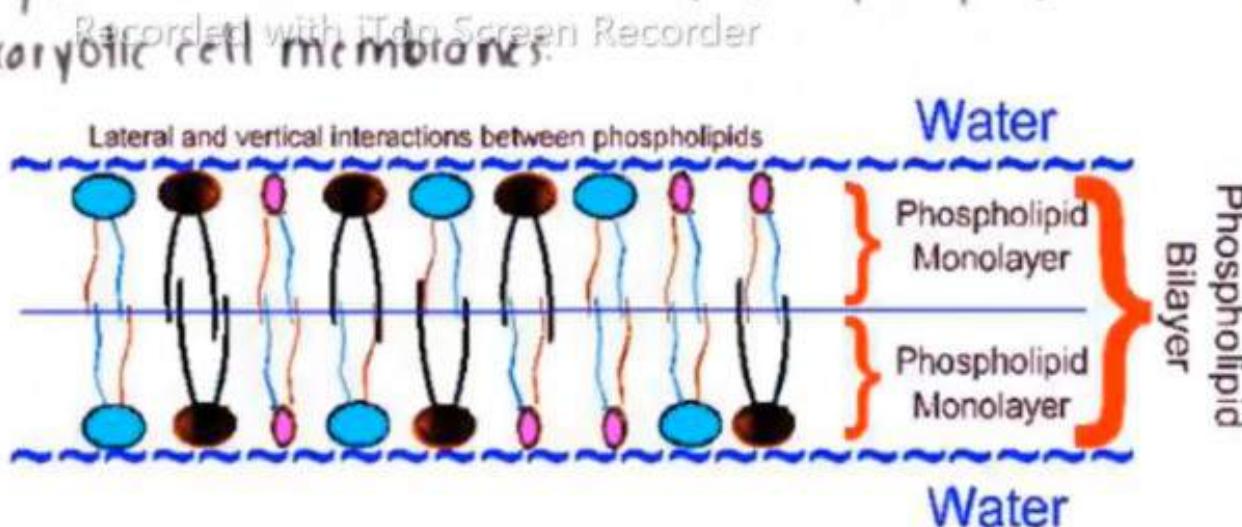
(c)



DOPC: -17°C

The structure of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)

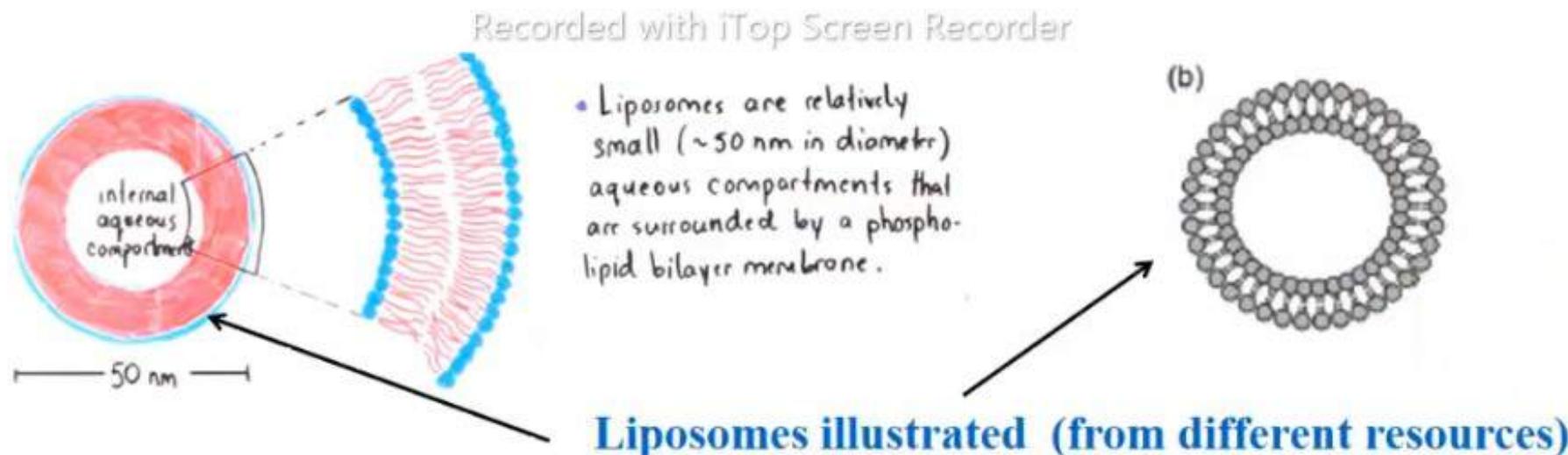
- Due to their amphiphilic nature, phospholipids have the ability to spontaneously form bilayer membranes. This is why phospholipids are major constituents of eukaryotic cell membranes.



Lipids Connection to Liposomes

- In addition, we can use the propensity of phospholipids to form membranes to create special structures called liposomes (also called lipid vesicles).

Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids.

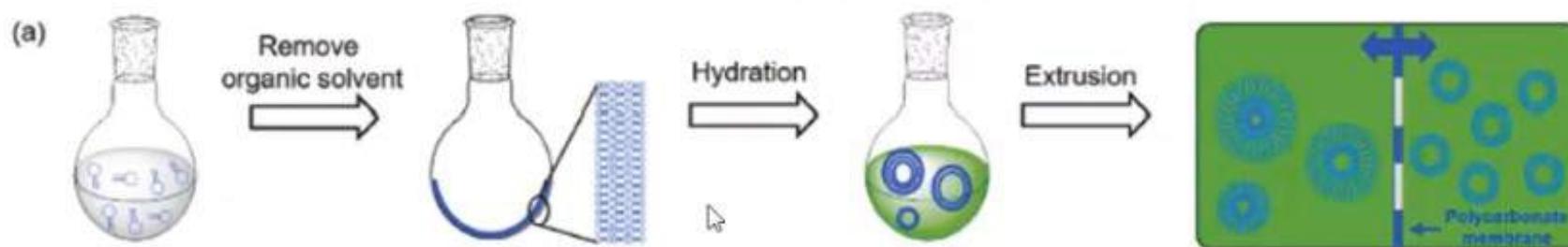


Formation of Liposomes

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Formation of Liposomes (Bangham Mtd)



(a) Schematic illustration of liposome preparation via hydration of dried lipid films followed by extrusion.

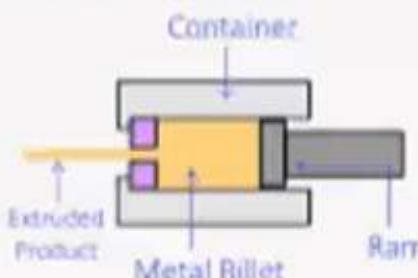
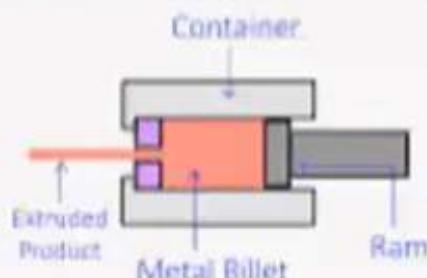
The most common Bangham method involves the formation of a lipid film by evaporating the organic solvent used to dissolve lipids.⁵⁴ After hydration with an aqueous solution, multilamellar vesicles with a heterogeneous size distribution are formed. By extrusion through a polycarbonate membrane, small unilamellar vesicles (SUVs) with a narrow size distribution are obtained (Fig. 2a). The extrusion temperature needs to be higher than the T_c of the lipids. This method can produce liposomes from ~ 50 nm to ~ 200 nm. The larger the membrane pores, the more likely to form multilaminar vesicles.

Extrusion

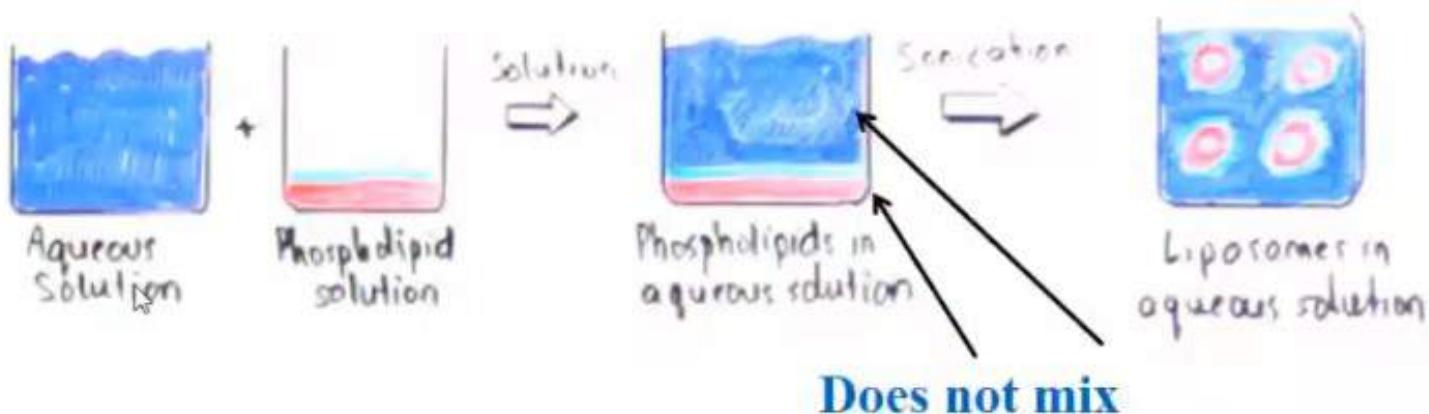
Extrusion is a technique where molten polymer is forced through a die and is used to produce components of a fixed cross-sectional area such as tubes and rods.

Hot Extrusion Cold Extrusion

Recorded with iTop Screen Recorder



Formation of Liposomes (Sonification)



Formation of Liposomes



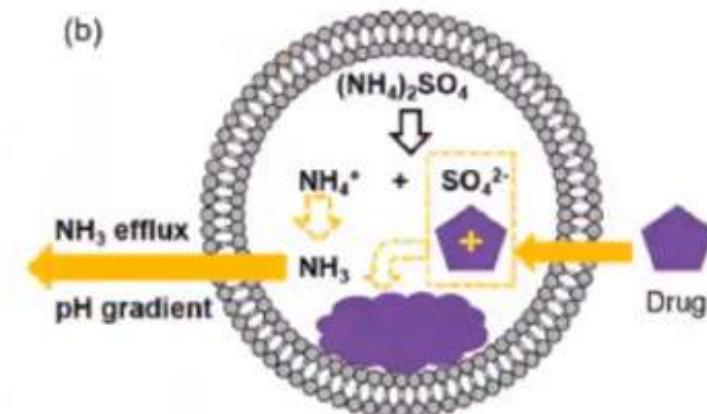
- Lipid vesicles can be formed by mixing a lipid solution into an aqueous solution and then sonicating the solution. Sonication involves bombarding the solution with sound waves. The energy carried by the sound waves disperses the lipids, allowing them to spontaneously aggregate into bilayer membranes (i.e. liposomes).



Formation of Liposomes (Active Drug loading)

Active drug loading, also known as remote loading, refers to loading drugs into preformed liposomes. Active loading usually takes advantages of diffusion properties when a pH gradient is established across lipid bilayers.⁵⁵ This method requires drugs to have both an uncharged form and a charged form, where only the uncharged drugs can cross liposome membranes. Once diffused into liposomes, they become charged and membrane-impermeable and entrapped inside. The remote loading method has led to the successful development of many commercial formulations.

Recorded with iTop Screen Recorder



For Doxil[®], a pH gradient

was generated by a transmembrane ammonium sulphate gradient. Ammonium salts could dissociate into ammonia and protons. Since ammonia has a high membrane diffusivity, a pH gradient can also be created. Thus, DOX can influx into liposomes and precipitate with ammonium counter ions remained inside to form membrane-impermeable drug complexes (Fig. 2b).⁵⁵

Classification of Liposomes

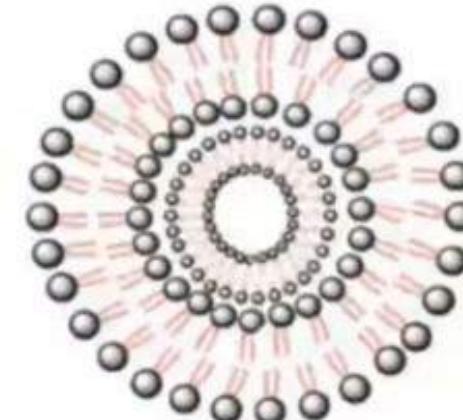
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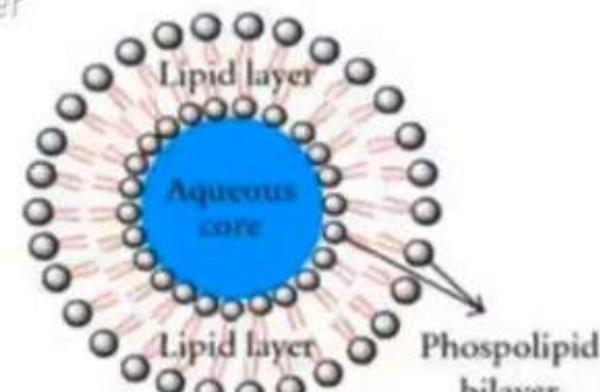
Classification of Liposomes

The **liposome size** can vary from very small ($0.025\text{ }\mu\text{m}$) to large ($2.5\text{ }\mu\text{m}$) vesicles. Moreover, liposomes may have

one or bilayer membranes. The **vesicle size** is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes. On the **basis of their size and number of bilayers**, liposomes can also be classified into one of two categories: (1) multilamellar vesicles (MLV) and (2) unilamellar vesicles. Unilamellar vesicles can also be classified into two categories: (1) large unilamellar vesicles (LUV) and (2) small unilamellar vesicles (SUV) [16]. In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water



Multilamellar liposomes



Unilamellar liposomes



Biophysical Properties of Liposomes for Drug delivery

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Biophysical Properties of Liposomes for Drug delivery

4.1 Liposome size

The size of liposomes is very important for drug delivery.⁶⁴

On one hand, it affects the blood circulating time of liposomes.

The clearance of nanoparticles and macromolecules in the bloodstream is mediated by the renal system, mononuclear phagocytic system (MPS) or reticuloendothelial system (RES). Since the estimated threshold for first-pass elimination by the kidneys is 10 nm, molecules and nanoparticles below 10 nm are rapidly eliminated by the renal system, while larger nanoparticles are mainly cleared by MPS.⁶⁵ On the other hand, vasculature in tumors is leaky due to enlarged endothelial pores, allowing nanoparticles with proper size to escape from the bloodstream and accumulate at tumor tissues rather than healthy organs. This is known as the enhanced permeability and retention (EPR) effect, although some recent work suggested that the EPR effect might not be as important in real tumors.



Biophysical Properties of Liposomes for Drug delivery

4.2 Liposome charge

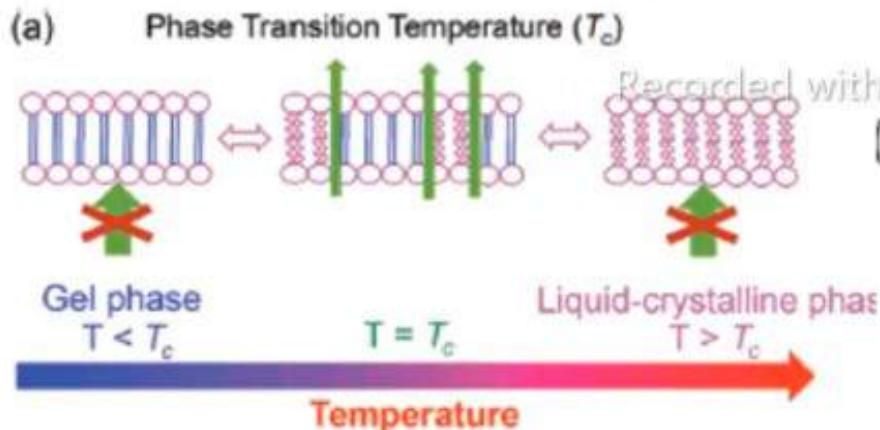
To achieve a long blood-circulation time, charge neutral zwitterionic PC liposomes are the most frequently used to reduce protein binding



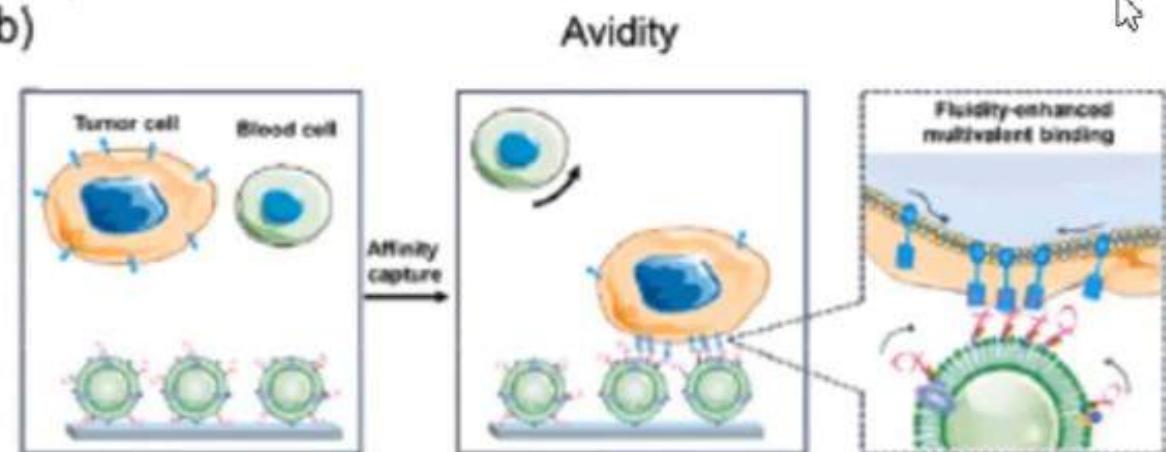
Biophysical Properties of Liposomes for Drug delivery

4.3 Liposome fluidity

The structure of lipid tails strongly influences the T_c of lipids, and controls their mechanical strength, lateral diffusion and permeability. The membrane permeability is the largest at T_c because of the coexistence and interconversion of the two phases, creating leaky phase boundaries (Fig. 3a).



Rearrangement of immobilized ligands allows for optimal polyvalent binding, increasing binding affinity (so called avidity for describing polyvalent interactions) (Fig. 3b).^{84,85} Tumor targeting by manipulating membrane fluidity was demonstrated in a recent work, with fluid liposomes preferentially targeting the tumor cells and gel-phase liposomes targeting the healthy cells.⁸⁶

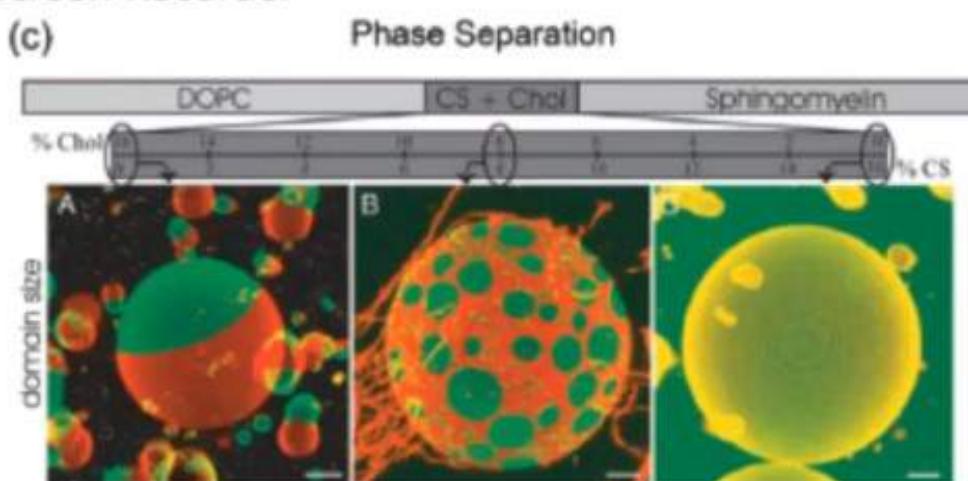


Biophysical Properties of Liposomes for Drug delivery

Another interesting property of lipid bilayers is their lateral organization. In addition to the liquid-crystalline phase and gel phases, phospholipids can form a liquid crystalline ordered phase (L_o) in the presence of cholesterol. Some ternary or four-component lipid mixtures can separate into distinct phases, a liquid-disordered (L_d) phase comprising mainly unsaturated lipid species, and a more packed liquid-ordered (L_o) phase enriched in saturated lipid and cholesterol.^{87,88} By incorporating fluorescent probes that selectively partition in the L_d or L_o phase, phase separation can be observed by confocal fluorescence microscopy (Fig. 3c).⁸⁷

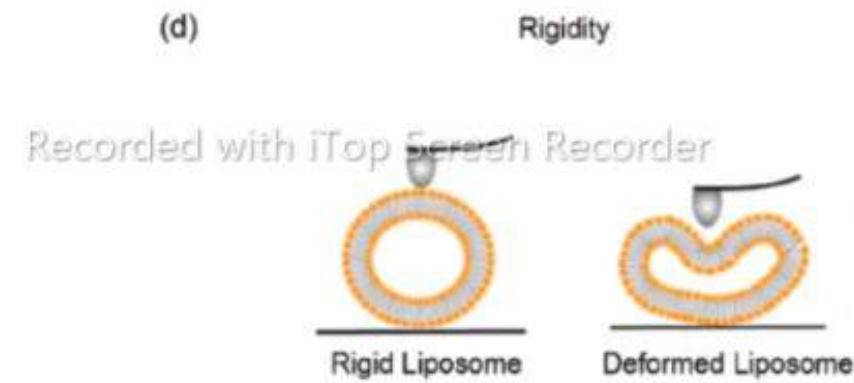
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(c) L_d/L_o phase



Biophysical Properties of Liposomes for Drug delivery

Another related factor that can be manipulated to facilitate drug delivery is lipid membrane rigidity or mechanical properties (Fig. 3d). Lipids with a higher T_c afford a more rigid structure with less deformability, while lipids with lower T_c are more flexible. Membrane rigidity influences both cellular uptake and liposome penetration in extracellular matrix (ECM) environment.



Desirable Characteristics of Drug Delivery System

Desirable Characteristics of Drug Delivery System

- *Suitable for delivery of hydrophobic, hydrophilic and amphipathic drugs and agents*
- *Reduce toxicity of encapsulated drug*
- *Non-toxic, flexible, biocompatible, completely biodegradable and non-immunogenic for systemic and non-systemic administration*
- **Biocompatible** Recorded with iTop Screen Recorder
- *Suitable for controlled release*
- *Suitable to give localized action in particular tissues.*
- *Flexibility to couple with site-specific ligands to achieve active targeting*

Liposomes and skin: From drug delivery to model membranes

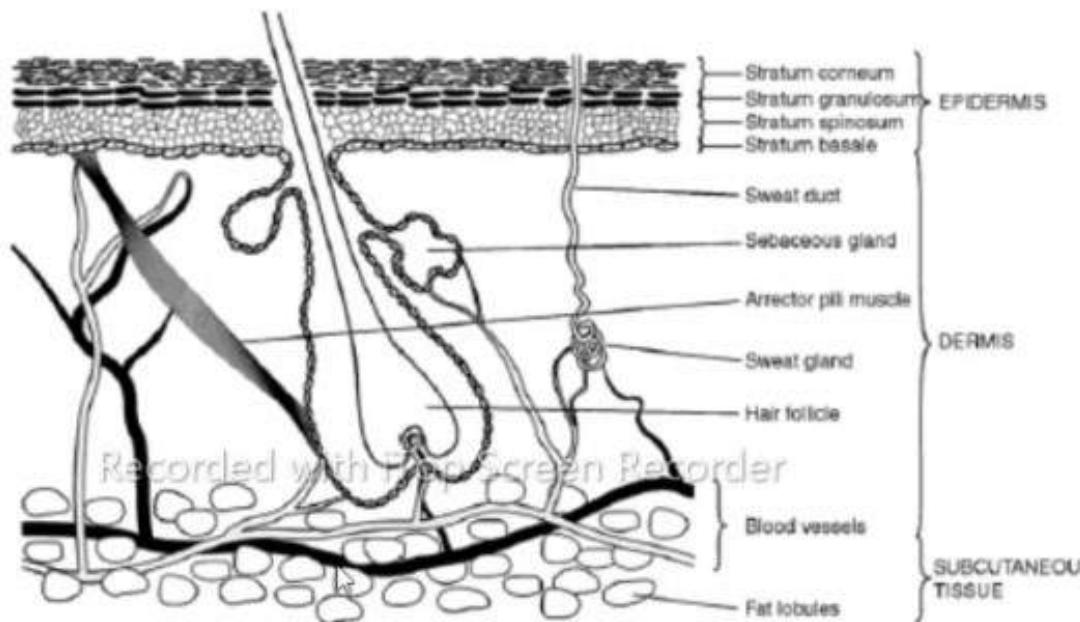


Fig. 1 – A diagrammatical representation of a cross-section through human skin showing the different cell layers and appendages (from Williams, 2003, with permission).

Liposomes and skin: From drug delivery to model membranes

The transepidermal pathway can be defined as the pathway where compounds permeate across the intact, unbroken stratum corneum. This pathway contains two micropathways. First, the intercellular route, which is a continuous but tortuous way through the intercellular lipid domains and secondly, the transcellular pathway through the keratinocytes, then across the intercellular lipids (Fig. 2) (Barry, 1991). As can be seen from Fig. 2, the transcellular pathway requires not only partitioning into and diffusion through the keratin bricks but also into and across the intercellular lipids. Thus, the intercellular lipids play a major role in the barrier nature of the SC.

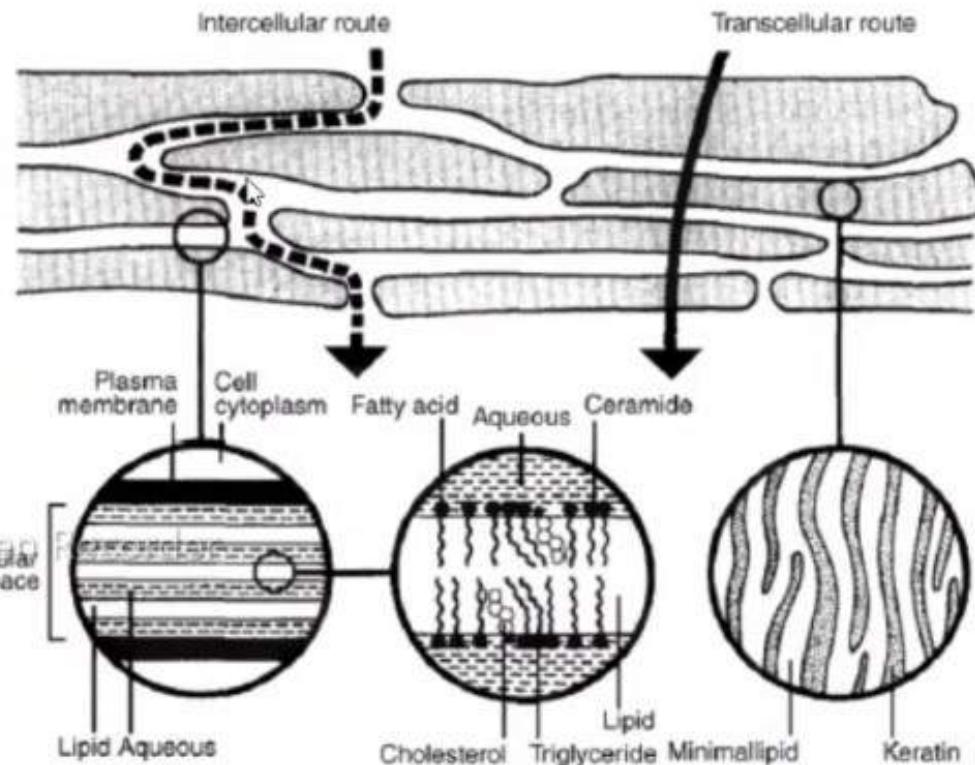


Fig. 2 – Diagram of the brick and mortar model of the stratum corneum with a simplified lamellar organization of intercellular domains showing the major stratum corneum lipids. Also shown are the possible drug permeation pathways through intact stratum corneum; the transcellular or the tortuous intercellular pathways (after Elias, 1981; Barry, 1991).

4. Mechanisms of action of liposomes as skin drug delivery systems

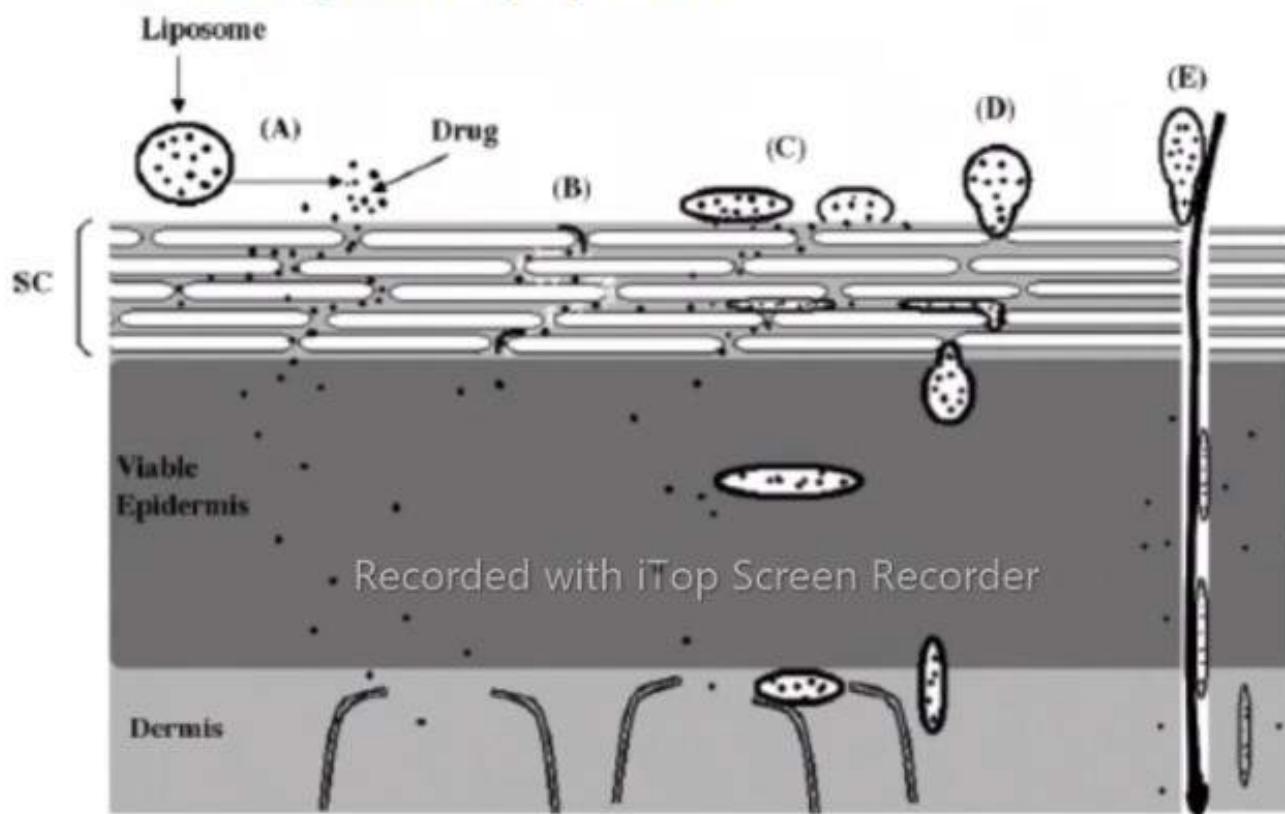


Fig. 3 – Possible mechanisms of action of liposomes as skin drug delivery systems. (A) is the free drug mechanism, (B) is the penetration enhancing process of liposome components, (C) indicates vesicle adsorption to and/or fusion with the stratum corneum (SC) and (D) illustrates intact vesicle penetration into or into and through the intact skin (not to scale) (modified from El Maghraby et al., 2006).

Table 2 – Summary of the specifications, advantages and limitations of various models of human skin employed in transdermal drug delivery research

Model	Specification/advantages	Limitations
Animal models Monkeys Wester and Maibach (1975), Wester et al. (1980)	Percutaneous absorption compares well to human skin	Restricted use and high cost
Rodents Bartek et al. (1972), Simon and Maibach (1998), Poet et al. (2000)	Relatively cheap and readily available. Rat may be the best rodent model. The problem of fur can be eliminated using hairless animals	More permeable than human skin

5.2. Liposomal models

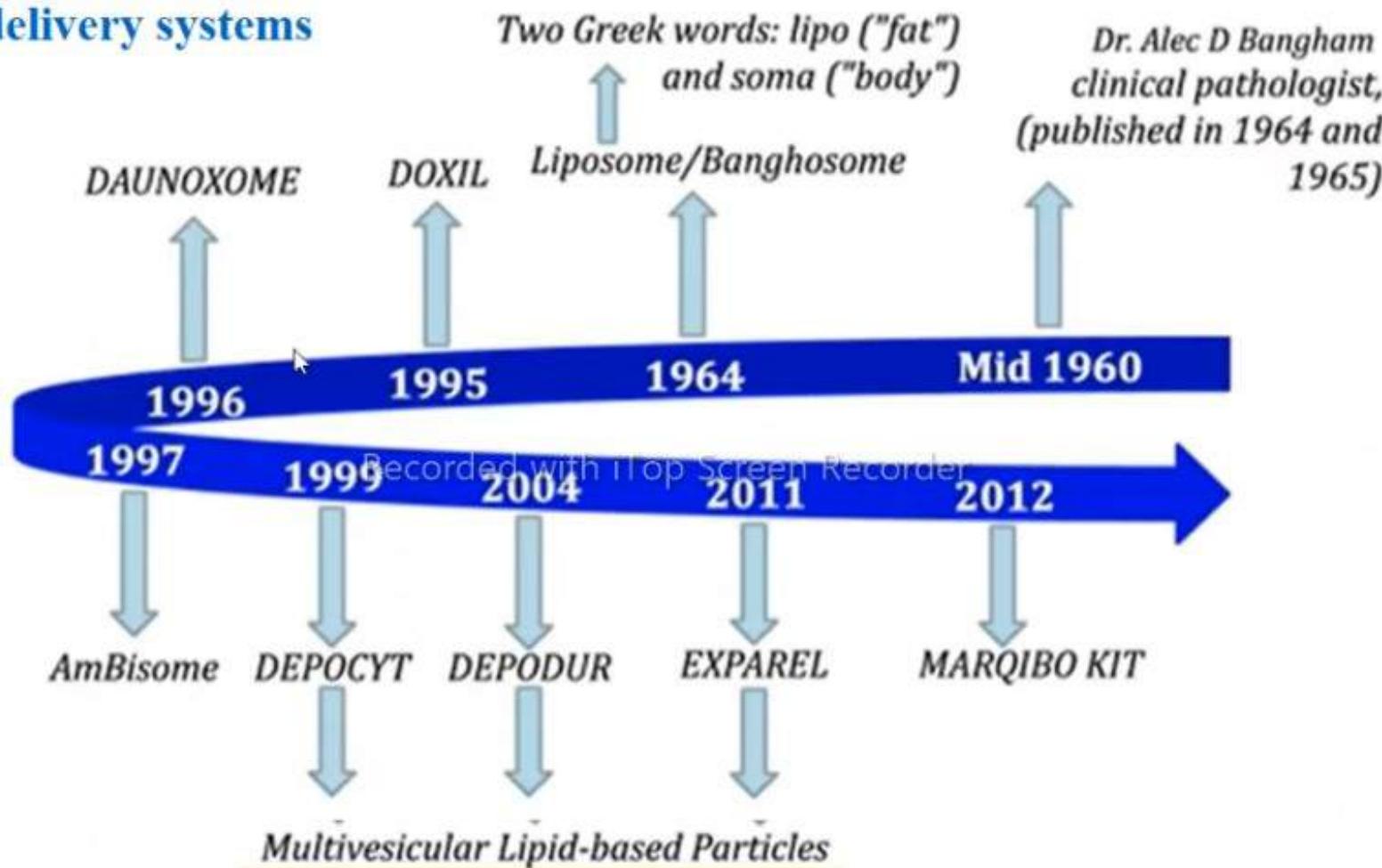
Since SC intercellular lipids form bilayers, liposomes have been proposed as models for skin membranes. Simple phospholipid liposomes comprising DPPC were initially used before Wertz et al. (1986) prepared liposomes from a lipid mixture approximating the lipid composition of the SC lipids (40% ceramides, 25% cholesterol, 25% palmitic acid and 10% cholesterol sulphate), termed SCL liposomes. These structures were used mainly to investigate the mechanisms of enhanced skin drug delivery. They were also used to investigate the possible oxidant or antioxidant effect of certain materials. This section will summarise these applications.

Liposomes offer potential value in dermal and transdermal drug delivery and recent advances and modifications appear to have generated increased therapeutic potential. Alteration in their composition and structure results in vesicles with tailored properties. Flexible and ultradeformable liposomes are one such advance with claims of enhanced transdermal drug delivery to efficiencies comparable with subcutaneous administration.



Breakthrough Technologies in Liposomes

Drug delivery systems



Benefits of Drug loading in Liposomes

The benefits of drug load in liposomes, which can be applied as (colloidal) solution, aerosol, or in (semi) solid forms, such as creams and gels, can be summarized into

Table 2 Benefits of drug load in liposomes

Benefits of drug load in liposome	Examples
1. Improved solubility of lipophilic and amphiphilic drugs	Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines, respectively; hydrophilic drugs, such as anticancer agent doxorubicin or acyclovir
2. Passive targeting to the cells of the immune system especially cells of the mononuclear phagocytic system	Antimonials, amphotericin B, porphyrins, vaccines, immunomodulators
3. Sustained release system of systemically or locally administered liposomes	Doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin
4. Site-avoidance mechanism	Doxorubicin and amphotericin B
5. Site-specific targeting	Anti-inflammatory drugs, anti-cancer, anti-infection
6. Improved transfer of hydrophilic, charged molecules	Antibiotics, chelators, plasmids, and genes
7. Improved penetration into tissues	Corticosteroids, anesthetics, and insulin



STRUCTURE AND DYNAMICS OF LIPID MONOLAYERS: THEORY AND APPLICATIONS

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journal homepage: www.elsevier.com/locate/bbamem

Review

Complementary biophysical tools to investigate lipid specificity in the interaction between bioactive molecules and the plasma membrane:
A review

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Papers/Books referred for Todays Lecture



Biomembrane frontiers : nanostructures, models, and the design of life

New York : Humana Press, ©2009.

1 online resource (xxii, 323 pages) : illustrations

[Handbook of modern biophysics ; v. 2.](#)



MODEL MEMBRANE –LIPID MONOLAYER

a. Introduction to Model Membrane

b. Lipid Monolayer in Biology

c. Structure and Dynamics of Lipid Monolayer

d. Modelling using Lipid Monolayer
Recorded with iTop Screen Recorder

e. Biophysical Methods for Lipid Monolayer

Acknowledgment: Aggregated Lecture from lot of resources
(Textbook/Journal Papers/Youtube lectures)

1.3. Models of membranes

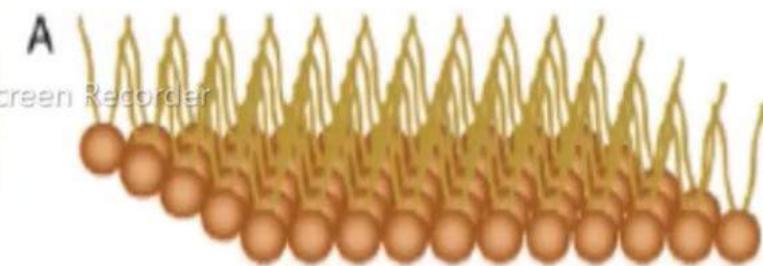
Introduction to Model Membranes

The existence of lipid domains, lipid asymmetry, coexistence of phases and diversity in lipid composition are the reasons why membranes are extremely intricate structures. The complexity of this system is further increased by their association with proteins and carbohydrates. This complexity makes the biophysical interactions with bioactive molecules very difficult to investigate in a 'real' situation [28]. Therefore, simplified artificial membrane systems, which mimic the natural bilayer lipid membrane, have been developed [28,29].

Model membranes are systems in which the organization best mimics the lipid arrangement found in natural plasma membranes. Three systems are widely used, i.e., lipid monolayers, supported bilayers and liposomes (Fig. 1). While each of these systems exhibits advantages and disadvantages, they all mimic to a certain extent the lipid arrangement of natural cell membranes (for review see [22]).

Lipid monolayers at the air/water interface offer excellent model systems for various areas in science. They can be used as models to study two-dimensional and surface phenomena in physics and chemistry, such as adsorption, surface activity, wetting, ordering, and phase transitions.

In biology, lipid monolayers represent models for biological membranes and biologically important interfaces, such as the gas exchange interface in the lungs and tear film in the eyes.



Introduction to Model Membranes

2. What kind of lipid to choose?

Since model membranes should be representative of natural membranes, their reconstitution must reflect this "real" composition as

best, taking practical and experimental details into account. It is hence important to soundly consider the choice of the lipids, of the model (monolayer vs bilayer, type of liposome,... see above) and of the complexity of the lipid mixture.

- **Choice of lipids:** This choice will be done according to different criteria. Recorded with iTop Screen Recorder The first one consists in choosing an appropriate lipid for the organism under consideration [76,77]. For example, concerning model sterol, it is better to choose Chol for a study with red blood cells [78,79], sitosterol when working with plants
 - **Choice of lipid mixture:** The lipid composition of the model membrane is one of the most important points. As expected, more the composition of the model membrane is close to the reality, more the experimental results will be biologically relevant. However, increasing the complexity of the model membrane will in turn render the biophysical observations difficult to interpret.
- Balance between biophysical observations and complexity of model membrane is important**

Lipid Monolayer in Biology

In biology, lipid monolayers represent models for biological membranes and biologically important interfaces, such as the gas exchange interface in the lungs and tear film in the eyes.

Lipid monolayers provide a simple model considered as half the bilayer of biological membranes. They can be used to study the ability of compounds to penetrate into the outer leaflet of the membrane and to characterize the interactions of the molecule of interest with lipids [30,31]. They are formed at the air–water interface of a Langmuir trough by spreading lipids of the membrane under consideration. Parameters such as the nature and the packing of the spread molecules, the composition of the subphase (pH, ionic strength) and temperature can be varied in a controlled way and without limitation [32].

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Lipid Monolayer: Molecular Density and Phase behaviour

The properties of lipid monolayers depend dramatically on their molecular density. They can form various phases in two dimensions which are controlled by temperature and by the surface pressure, analogous to the three-dimensional pressure [5].

Lipid monolayers at the air/water interface can adopt different molecular densities, depending on the amount of lipid and on the available interfacial area. The molecular density can be changed by varying the monolayer area at the interface or by applying an external surface pressure. The monolayer molecular density in turn determines the surface tension at the interface.

tension at the interface in the presence of lipid molecules, i.e., the surface tension in the monolayer, γ_m , depends on the surface pressure, $\Pi(A_L)$, and the surface tension at the bare interface, γ_0 :

$$\gamma_m(A_L) = \gamma_0 - \Pi(A_L), \quad (3.1)$$

where A_L denotes the area per lipid (inversely proportional to monolayer molecular density).

Dynamics of Lipid Monolayer

Dynamic properties of lipid monolayers can be evaluated using time correlation functions. The (long-range) lateral diffusion coefficient, D , of lipid molecules is given by

$$\langle r^2(t) \rangle = 4 \cdot D \cdot t, \quad \text{Recorded with iTop Screen Recorder} \quad (3.3)$$

where $r(t)$ is the lateral displacement of molecules as a function of time. Rotational motions of lipids around their molecular axis can be described by a rotational autocorrelation function for the groups of interest.

Dynamics of Lipid Monolayer

Understanding Correlation Function

All correlation-based approaches involve taking the sum of the sample-by-sample product of the two functions:

$$\begin{aligned} r_{xy} &= x[1]y[1] + x[2]y[2] + x[3]y[3] + \dots + x[N]y[N] \\ &= \sum_{n=1}^N x_n y_n \end{aligned} \quad \text{Recorded with iTop Screen Recorder} \quad (2.29)$$

where r_{xy} is used to indicate correlation and the subscripts x and y indicate what is being correlated. Equation 2.29 uses the summation of the sample-by-sample product, but often the mean of the summation is taken, as

$$r_{xy} = \frac{1}{N} \sum_{n=1}^N x_n y_n \quad (2.30)$$

Dynamics of Lipid Monolayer

Monolayer phase behavior can be best described using a surface pressure-area isotherm. A schematic representation of the isotherm for a single component lipid monolayer at a temperature below the main phase transition temperature, T_m , is shown in Figure 3.3a. The main phase transition temperature corresponds to the transition from the liquid-crystalline to the gel phase in the bilayer of the same composition (at the normal atmospheric pressure).

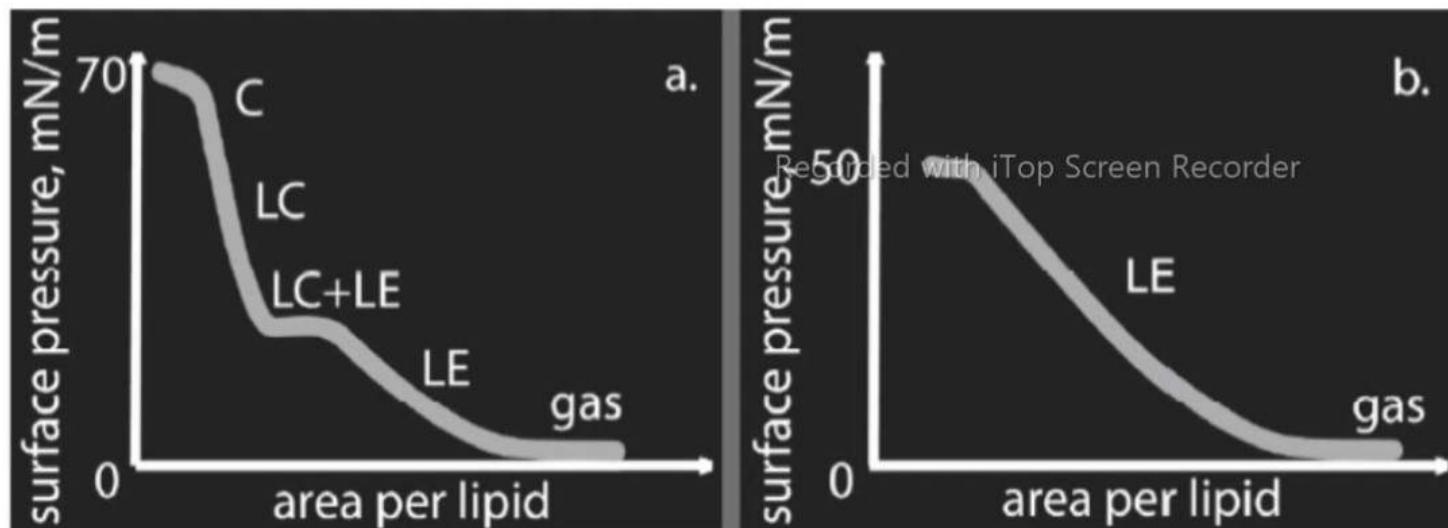


Figure 3.3. Schematic surface pressure-area isotherm of a lipid monolayer at temperatures (a) below and (b) above the main phase transition temperature.

LE- Liquid Expanded phase; LC-Liquid Crystalline

Dynamics of Lipid Monolayer

Figure 3.4. Fluorescence microscopy images of a lipid monolayer (DPPC and POPG in ratio 4:1 at 25°C): (a) coexistence of LE and gas phases; (b) coexistence of LC and LE phases. Please visit

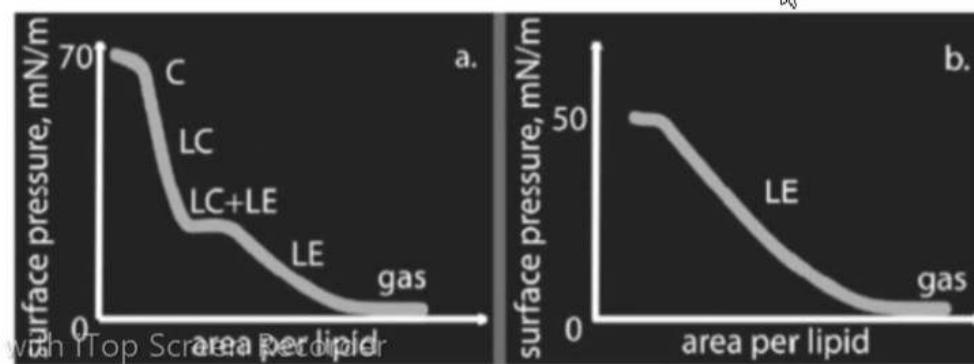
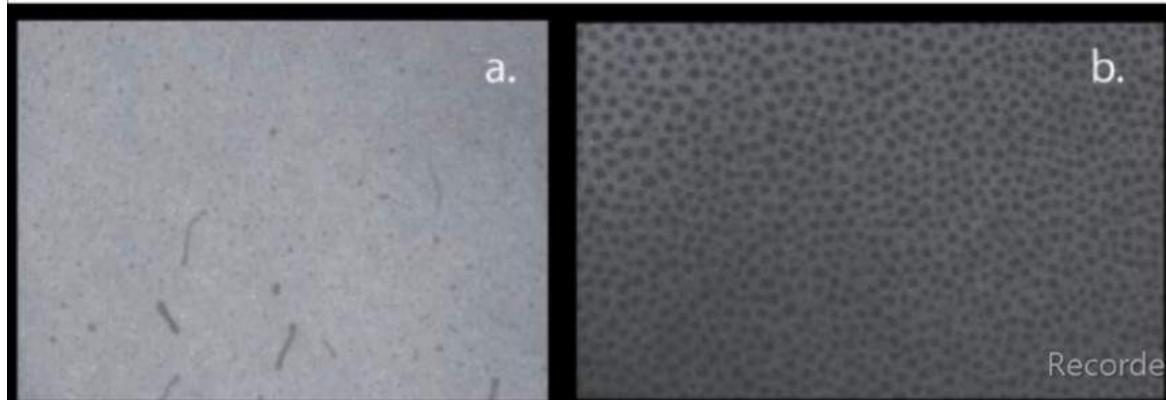


Figure 3.3. Schematic surface pressure-area isotherm of a lipid monolayer at temperatures (a) below and (b) above the main phase transition temperature.

At smaller molecular areas, the gas phase coexists with the liquid-expanded (LE) phase. The structure of the coexisting phases is very heterogeneous; the domains of the two phases assume different shapes, with the gas phase forming “bubbles” in the LE phase (see Fig. 3.4a). In this region the surface pressure is very low.

In the plateau part of the isotherm the monolayer undergoes a first-order phase transition from the LE into the LC phase. Each point in this region corresponds to the coexistence of the two phases (Fig. 3.4b).

Modelling using Lipid Monolayer

Lipid monolayers can be used as models to study complex phenomena in biological systems. For example, lipid monolayer constitutes the outer layer of tear film in the eyes [22]. Lipid monolayer is the main structural element of lung surfactant, which has very low protein content.

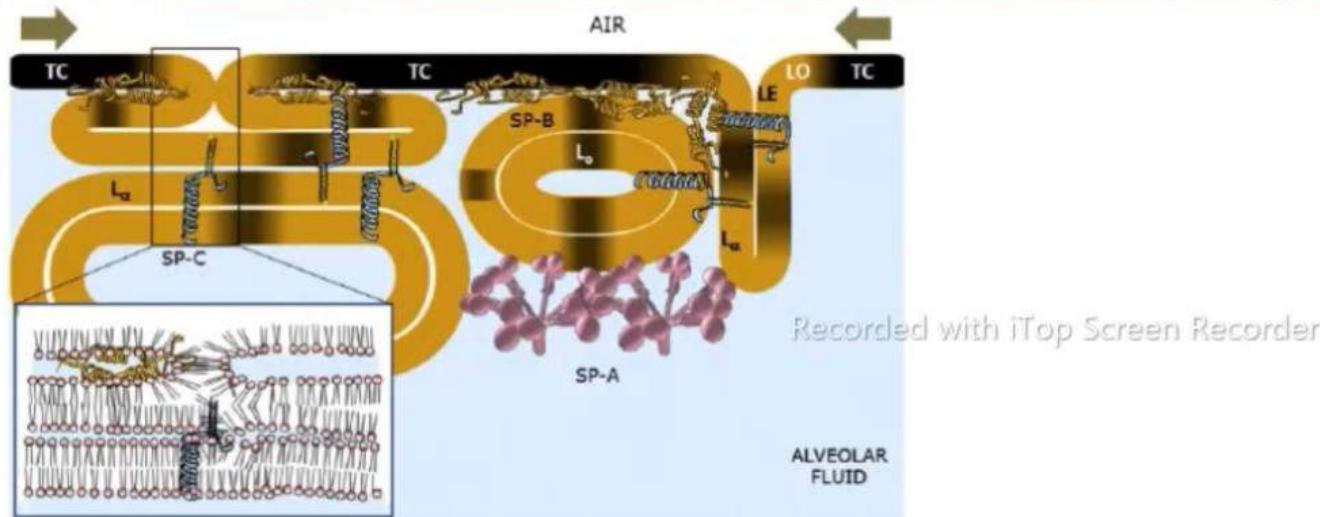
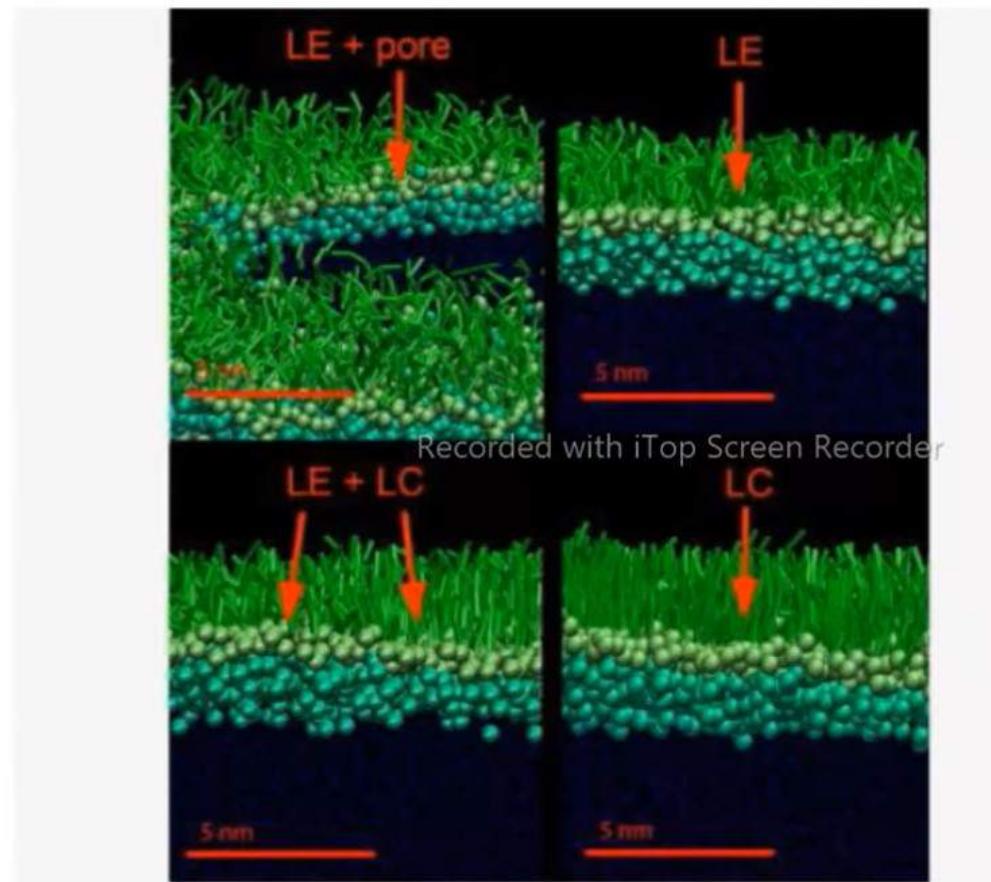


Fig1. 3D structure of lung surfactant.

Lung surfactant is a mixture of lipids and proteins forming a monomolecular film at the gas exchange interface in the lung alveoli [8]. Lung surfactant facilitates breathing. Its deficiency or inhibition causes failure of lung function that leads to severe respiratory disorders.

Lung surfactant consists mainly of PC lipids (80% by weight), with DPPC as major component (40% w). Anionic PG and PI lipids account for 8–15% by weight. Lung surfactant also contains cholesterol (5–10% w), PE lipids, fatty acids, and other minor components. Overall, lung surfactant is characterized by a rather complex lipid composition,

Modelling using Lipid Monolayer

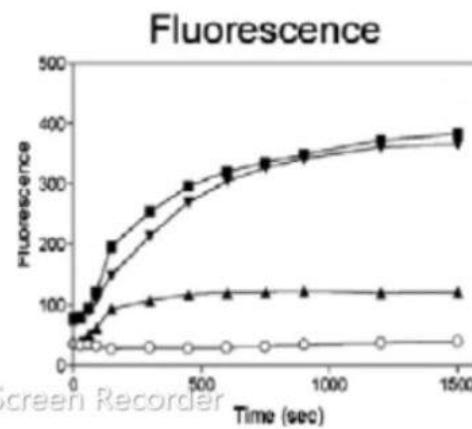


LE, and LC phases and their coexistence in a DPPC monolayer.

Biophysical Methods to Gather info on Lipids

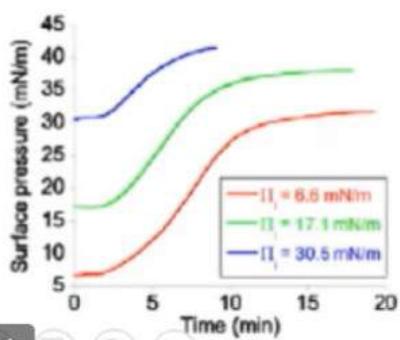
We have divided the biophysical methods into two groups (that are non-exhaustive), depending on the kind of information you get:

1. "Global molecular" methods giving information on the global effects of the molecule of interest on the lipids. These include (among others): Langmuir monolayer technique, isothermal titration calorimetry (ITC), fluorescence spectroscopy and imaging, atomic force microscopy (AFM), neutron reflectivity (NR), surface plasmon resonance (SPR) or electron paramagnetic spectroscopy (EPR).



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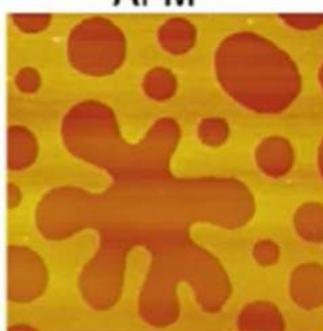
Langmuir through



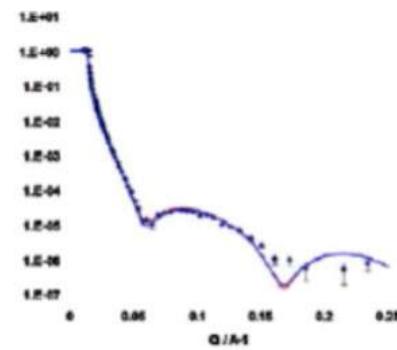
Cota Navin Gupta



AFM

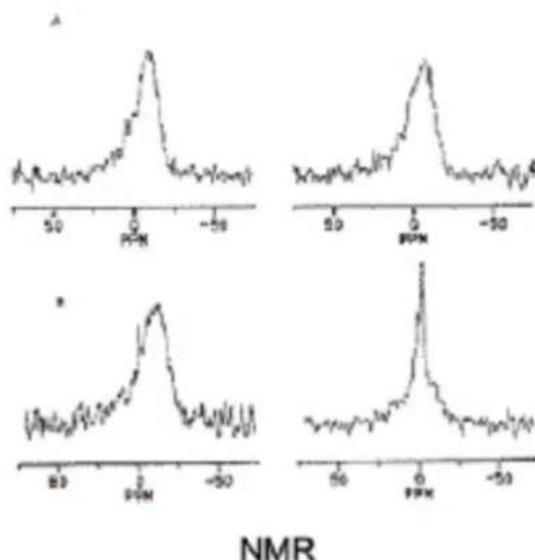


Neutron reflectivity

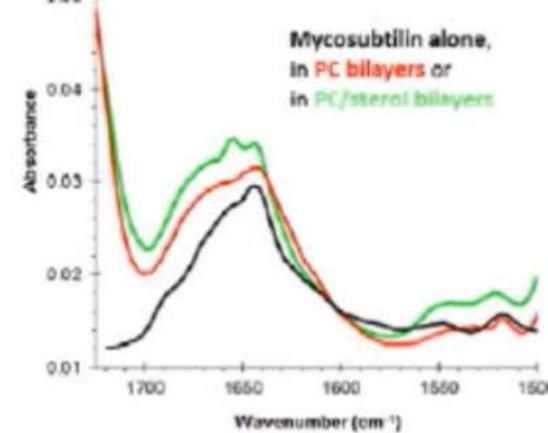


Biophysical Methods to Gather info on Lipids

In the second group, we have considered techniques giving information at the molecular/atomic level, that we call “molecular-specific” techniques. In that group, we gathered infrared spectroscopy (FTIR and PM-IRRAS), nuclear magnetic resonance (NMR) on lipids (^{31}P , ^2H , ^{13}C NMR), secondary ion mass spectrometry (SIMS) and “in silico” approaches.

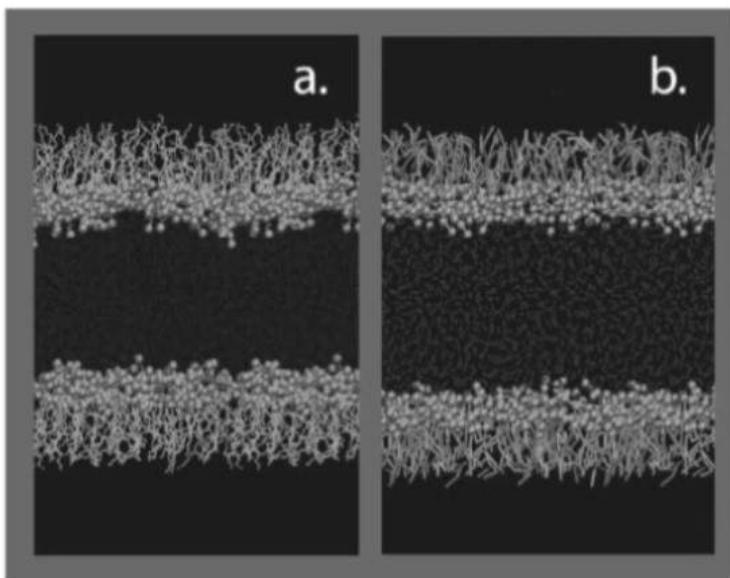


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IR spectroscopy

Lipid Monolayer in Atomic Detail



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DPPC monolayers at the vacuum/water interfaces in (a) atomistic :

A DPPC monolayer is one of the most extensively studied systems. In simulations, the properties and phase behavior for a DPPC monolayer have been investigated using various all-atom models [38–41]. An atomistic representation of the monolayer is shown in Figure 3.6a. It has

Recap

Good evening Sir,

I had a doubt in the lipid monolayer density equation.

According to the paper, it states that the surface tension at the interface in the presence of lipid molecules, i.e., the surface tension in the monolayer, γ_m , depends on the surface pressure, $\pi(A)$, and the surface tension at the bare interface, γ_0 :

$$\gamma_m(A) = \gamma_0 - \pi(A)$$

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In this γ_0 has the unit of tension, $\pi(A)$ has the unit of pressure and $\gamma_m(A)$ has tension \times area per lipid unit.

So mathematically how this equation is possible. As for any quantity to be added or subtracted all should be of same dimensions.

Recap

tension at the interface in the presence of lipid molecules, i.e., the surface tension in the monolayer, γ_m , depends on the surface pressure, $\Pi(A_l)$, and the surface tension at the bare interface, γ_0 :

$$\gamma_m(A_l) = \gamma_0 - \Pi(A_l), \quad (3.1)$$

where A_l denotes the area per lipid (inversely proportional to monolayer molecular density).

I guess you are referring to eqn 3.1 on page 4 in the uploaded chapter on monolayer lipids..If you read closely the content on attached slide

The left side of eqn is surface tension in monolayer and the right has surface pressure and surface tension at the bare interface

Surface pressure can be interpreted as below in the link

<https://www.sciencedirect.com/topics/chemistry/surface-pressure>

According to the link π is difference in interfacial tension.

So does it means that A which is the area per lipid is not being multiplied to π and γ_m rather it shows that those are function of A (area per lipid).

Surface Pressure

The surface pressure (Π) is defined as the difference in interfacial tension between a clean interface and an interface in the presence of emulsifier: $\Pi = \gamma_0 - \gamma$.

Websites

<https://www.nanoscience.com/techniques/scanning-tunneling-microscopy/>

<https://www.nanoscience.com/techniques/atomic-force-microscopy/>

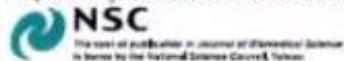


Imaging modes of atomic force microscopy for application in molecular and cell biology

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Yves F. Dufrêne^{1*}, Toshio Ando², Ricardo Garcia³, David Alsteens¹, David Martinez-Martin⁴, Andreas Engel⁵, Christoph Gerber⁶ and Daniel J. Müller^{4*}

Ramana et al. *Journal of Biomedical Science* 2010, 17:57
<http://www.jbiomedsci.com/content/17/1/57>



RESEARCH

Development of a liposomal nanodelivery system for nevirapine

Journal Papers referred for Todays Lecture



Open Access

Biophysical Methods to Gather info on Lipids



- a. Introduction/Principle of Scanning Tunnel Microscopy (STM)
- b. STM application on Liposomes
- c. Need for Atomic Force Microscopy (AFM)
- d. Applications of AFM

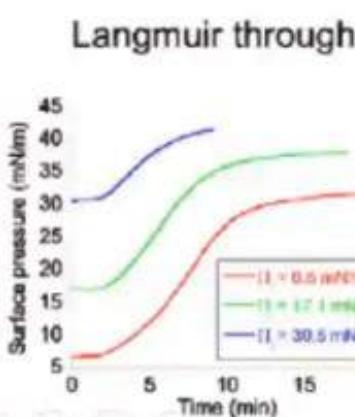
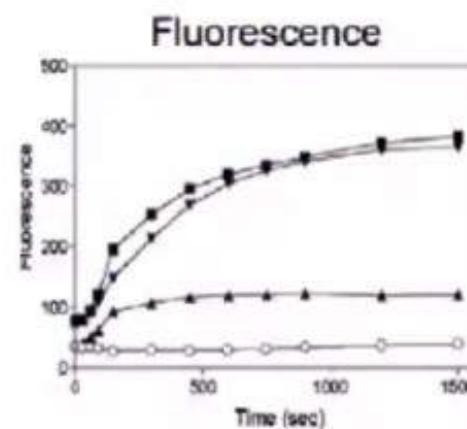
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Acknowledgment: Aggregated Lecture from lot of resources
(Textbook/Journal Papers/Youtube lectures)

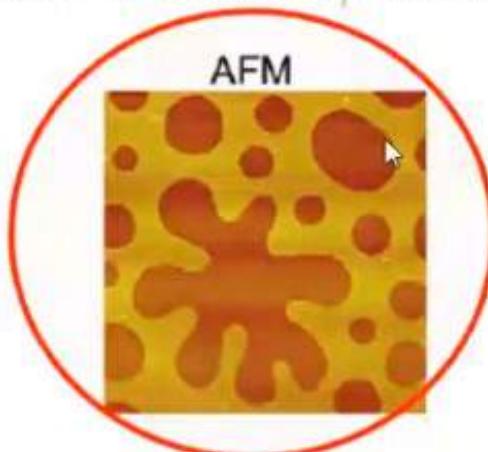
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Introduction to Scanning Tunnel Microscope

The development of the family of scanning probe microscopes started with the original invention of the STM in 1981. Gerd Binnig and Heinrich Rohrer developed the first working STM while working at IBM Zurich Research Laboratories in Switzerland. This instrument would later win Binnig and Rohrer the Nobel prize in physics in 1986.

How an STM Works

The scanning tunneling microscope (STM) works by scanning a very sharp metal wire tip over a surface. By bringing the tip very close to the surface, and by applying an electrical voltage to the tip or sample, we can image the surface at an extremely small scale – down to resolving individual atoms.

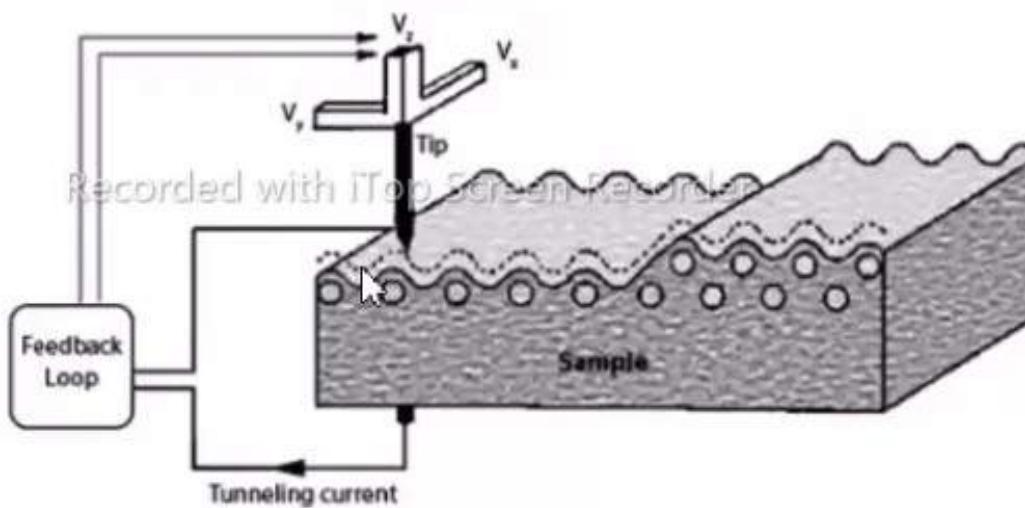
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3D rendered Scanning Tunneling Microscope image of atoms.

Principle of Scanning Tunnel Microscope

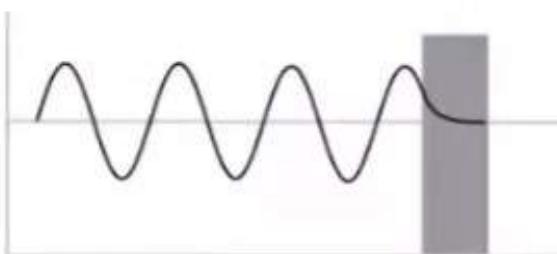
The STM is based on several principles. One is the quantum mechanical effect of tunneling. It is this effect that allows us to "see" the surface. Another principle is the piezoelectric effect. It is this effect that allows us to precisely scan the tip with angstrom-level control. Lastly, a feedback loop is required, which monitors the tunneling current and coordinates the current and the positioning of the tip. This is shown schematically below where the tunneling is from tip to surface with the tip rastering with piezoelectric positioning, with the feedback loop maintaining a current setpoint to generate a 3D image of the electronic topography:



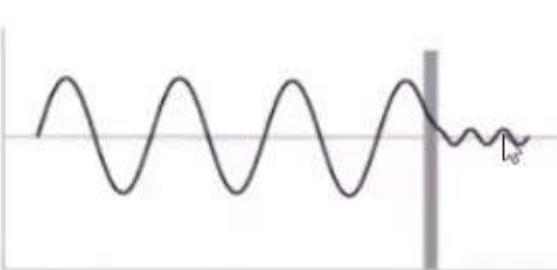
Schematic of scanning tunneling microscopy (STM)

Tunnelling in STM

Tunneling is a quantum mechanical effect. A tunneling current occurs when electrons move through a barrier that they classically shouldn't be able to move through. In classical terms, if you don't have enough energy to move "over" a barrier, you won't. However, in the quantum mechanical world, electrons have wavelike properties. These waves don't end abruptly at a wall or barrier, but taper off quickly. If the barrier is thin enough, the probability function may extend into the next region, through the barrier! Because of the small probability of an electron being on the other side of the barrier, given enough electrons, some will indeed move through and appear on the other side. When an electron moves through the barrier in this fashion, it is called tunneling.



The top image shows us that when an electron (the wave) hits a barrier, the wave doesn't abruptly end, but tapers off very quickly – exponentially. For a thick barrier, the wave doesn't get past.



The bottom image shows the scenario if the barrier is quite thin (about a nanometer). Part of the wave does get through and therefore some electrons may appear on the other side of the barrier.

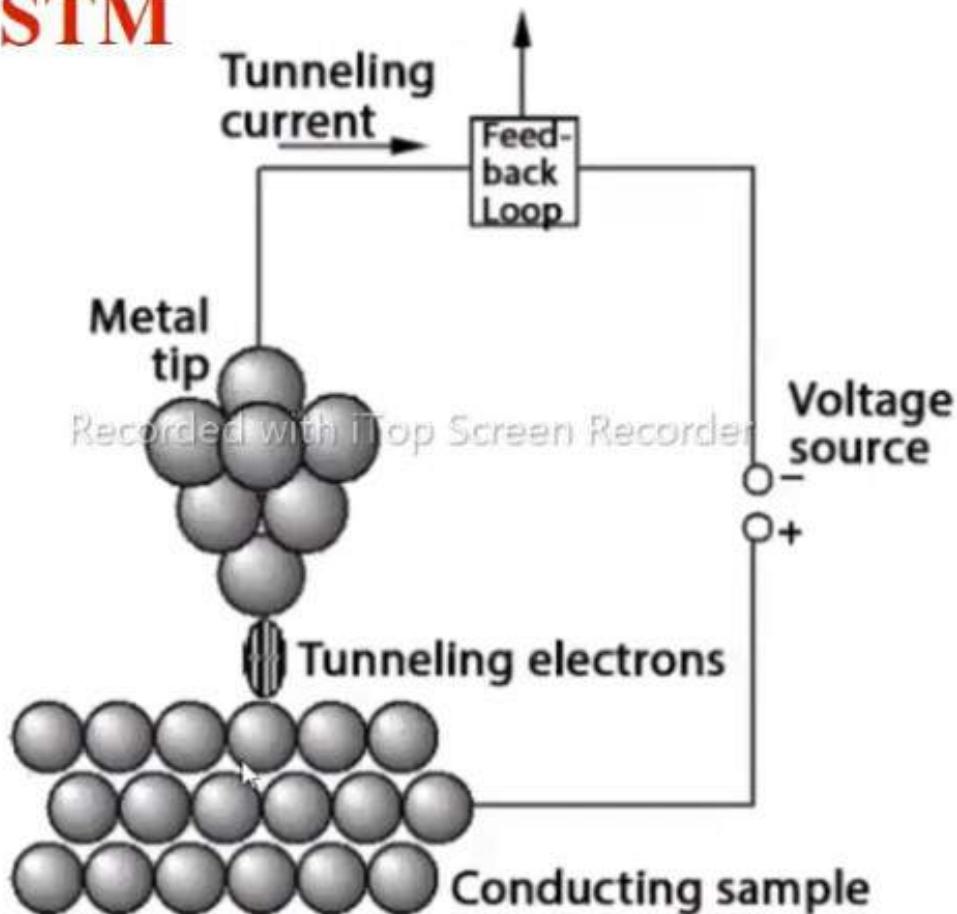
Because of the sharp decay of the probability function through the barrier, the number of electrons that will actually tunnel is very dependent upon the thickness of the barrier. The current through the barrier drops off exponentially with the barrier thickness.

Schematic of electron wavefunction.



To extend this description to the STM: The starting point of the electron is either the tip or sample, depending on the setup of the instrument. The barrier is the gap (air, vacuum, liquid), and the second region is the other side, i.e. tip or sample, depending on the experimental setup. By monitoring the current through the gap, we have very good control of the tip-sample distance.

Tunnelling in STM



STM-Other features

Piezoelectric Effect

The piezoelectric effect was discovered by Pierre Curie in 1880. The effect is created by squeezing the sides of certain crystals, such as quartz or barium titanate. The result is the creation of opposite charges on the sides. The effect can be reversed as well; by applying a voltage across a piezoelectric crystal, it will elongate or compress.

These materials are used to scan the tip in an scanning tunneling microscopy (STM) and most other scanning probe techniques. A typical piezoelectric material used in scanning probe microscopy is PZT (lead zirconium titanate).

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Feedback Loop

Electronics are needed to measure the current, scan the tip, and translate this information into a form that we can use for STM imaging. A feedback loop constantly monitors the tunneling current and makes adjustments to the tip to maintain a constant tunneling current. These adjustments are recorded by the computer and presented as an image in the STM software. Such a setup is called a constant current image.



STM Application on Liposomes

Preparation of Liposomes

Egg phospholipids were extracted from yellow yolk by the modified Singleton-Gray method [37]. The lipid composition of egg phospholipids have been identified using the GC-MS (Agilent technologies, Model 7890 A series, GC with 5975C Mass spectrometer). The results indicate that the egg phospholipid contains three different lipid constituents such as PLPC (89%), POPE (3%) and cholesterol (6%). Liposomes were prepared using the thin film hydration technique.

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Drug Loading

Nevirapine loaded liposomes were prepared dissolving eight different ratios of drug to phospholipids (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:10). Briefly, a total amount of lipid consisting of 10 mg of phospholipids in chloroform and different quantities of nevirapine was dissolved in chloroform and the liposomes were prepared as explained above.

STM Application on Liposomes

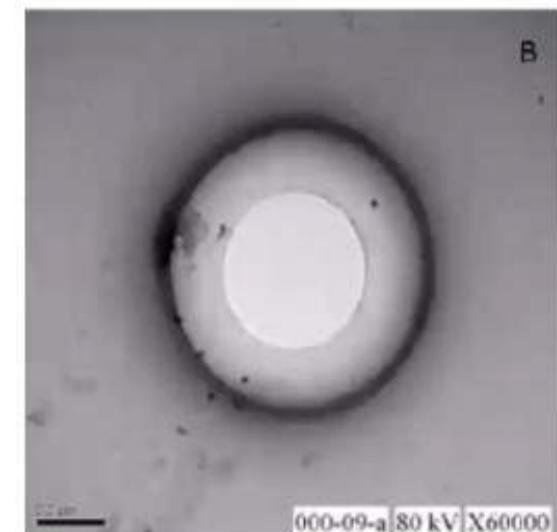
Results & Discussion

The mean particle size of the liposomes prepared using thin film hydration technique was 157 nm. The scanning electron micrograph of the lyophilized liposome indicates a spherical morphology and size in the nanodimensions (Figure 1A). Figure 1B presents the transmission electron micrograph of the liposomes clearly demarcating an aqueous phase in the centre of the liposome. The average aqueous volume of the liposomes determined from the various transmission electron micrographs is 15.54%. This small aqueous volume is likely due to the small vesicle sizes obtained in which considerable volume is occupied by the membrane [40].

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Figure 1 SEM and TEM micrographs of the lyophilized liposomes.

(A) The sample was imaged performed at a magnification of 43,000. Inset shows the magnified image of spherical liposome (B) TEM micrographs of liposome at a magnification of 60,000.



STM Application on Liposomes

Since lipid composition could have significant impact on liposome size, stability, drug loading and delivery functions, we examined the physical properties of the liposomes synthesized at varying ratios of egg phospholipids and cholesterol. The encapsulation efficiency of liposomes constituted from varying concentrations of phospholipid and cholesterol for nevirapine loading was compared (Figure 3). The encapsulation efficiency of the liposomes was significantly influenced by the presence of cholesterol and its drug to lipid ratio. Liposomes consisting of cholesterol (at 9:1 ratio) showed significantly increased encapsulation efficiency for nevirapine as compared to the particles without cholesterol (at 10:0 ratio, $p < 0.05$).

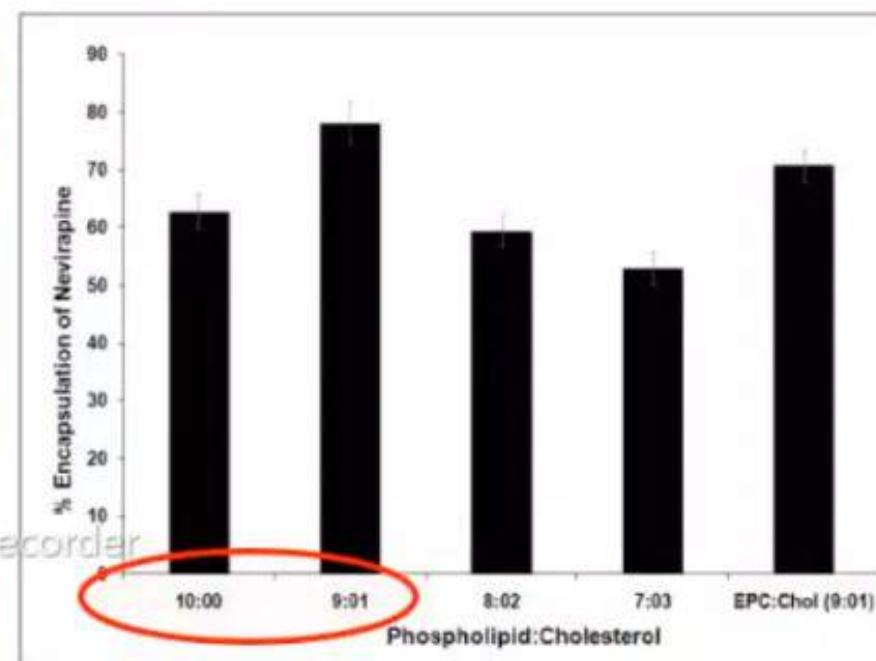


Figure 3 Effect of Cholesterol on the encapsulation efficiency of nevirapine. Statistical data infers that each group is significantly different ($p < 0.05$).

STM Application on Liposomes

We measured the effect of the drug-lipid ratio on liposome diameter and encapsulation efficiency. The results demonstrate that the size of the drug loaded liposomes decreased significantly from 188 ± 1.2 nm to 73 ± 7.8 nm when the drug-lipid ratio was decreased from 1:10 to 1:1 (Figure 4). A reduction in the liposome size is expected to shrink the aqueous volume of the liposome resulting in lesser encapsulation of a lipophilic drug like nevirapine [47]. Furthermore, significant increase in the amount of drug loading was observed with increasing drug-lipid ratio up to 1:5 ($p < 0.05$) but not beyond these ratio (Figure 4).

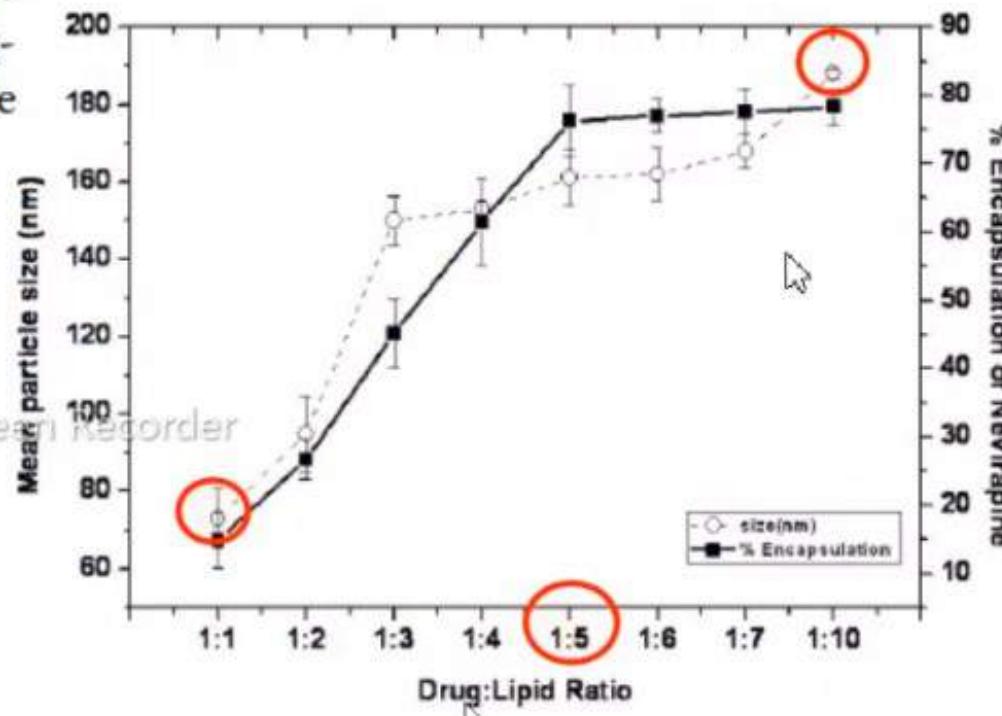


Figure 4 Correlation between the mean particle size (white circle) and percent encapsulation efficiency (Black square) at various drug-lipid ratios.

Need for Atomic Force Microscopy (AFM)

Topography imaging alone does not always provide the answers that researchers need and the surface topology often does not correlate to the material properties. For these reasons, advanced imaging modes have been developed to provide quantitative data on a variety of surfaces. Now, many material properties can be determined with AFM techniques, including friction, electrical forces, capacitance, magnetic forces, conductivity, viscoelasticity, surface potential, and resistance.



Working of Atomic Force Microscopy (AFM)

Analogous to how an Scanning Tunneling Microscope works, a sharp tip is raster-scanned over a surface using a feedback loop to adjust parameters needed to image a surface. Unlike Scanning Tunneling Microscopes, the Atomic Force Microscope does not need a conducting sample. Instead of using the quantum mechanical effect of tunneling, atomic forces are used to map the tip-sample interaction.

Often referred to as scanning probe microscopy (SPM), there are Atomic Force Microscopy techniques for almost any measurable force interaction – van der Waals, electrical, magnetic, thermal. For some of the more specialized techniques, modified tips and software adjustments are needed.

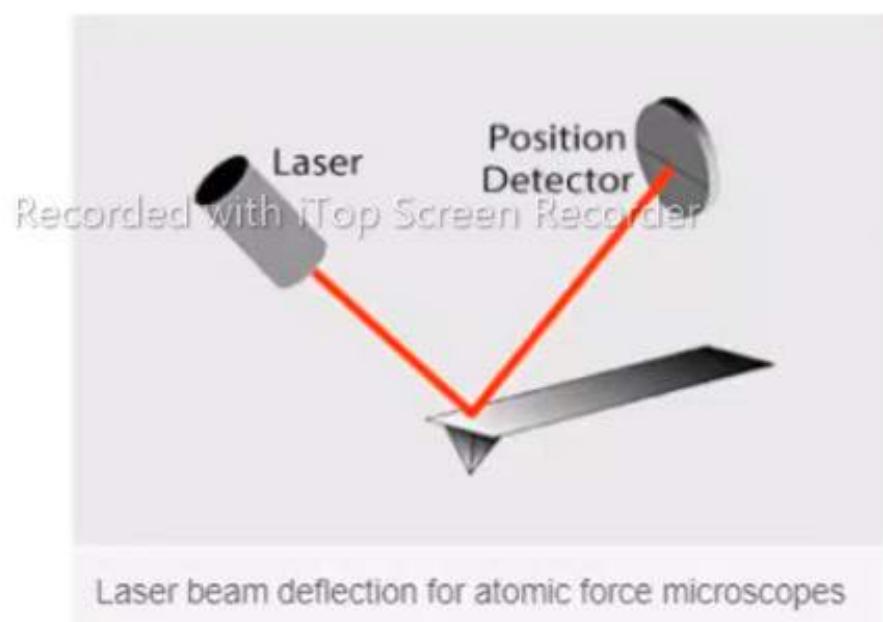
In addition to Angstrom-level positioning and feedback loop control, there are 2 components typically included in Atomic Force Microscopy: Deflection and Force Measurement.



AFM Probe Deflection

AFM Probe Deflection

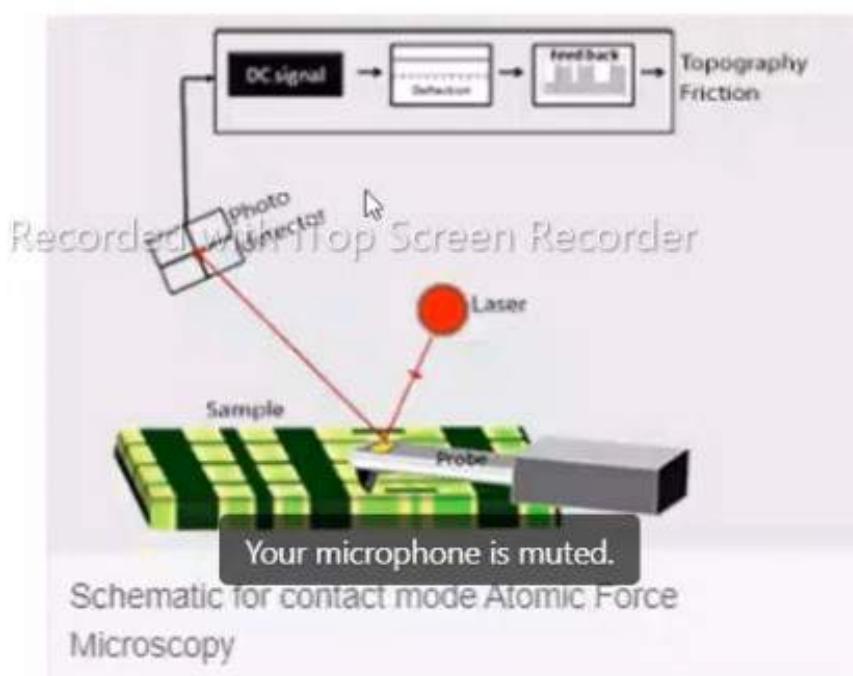
Traditionally, most Atomic Force Microscopes use a laser beam deflection system where a laser is reflected from the back of the reflective AFM lever and onto a position-sensitive detector. AFM tips and cantilevers are typically micro-fabricated from Si or Si_3N_4 . Typical tip radius is from a few to 10s of nm.



Feedback loop for AFM

Feedback Loop for Atomic Force Microscopy

Atomic Force Microscopy has a feedback loop using the laser deflection to control the force and tip position. As shown, a laser is reflected from the back of a cantilever that includes the AFM tip. As the tip interacts with the surface, the laser position on the photodetector is used in the feedback loop to track the surface for imaging and measuring.



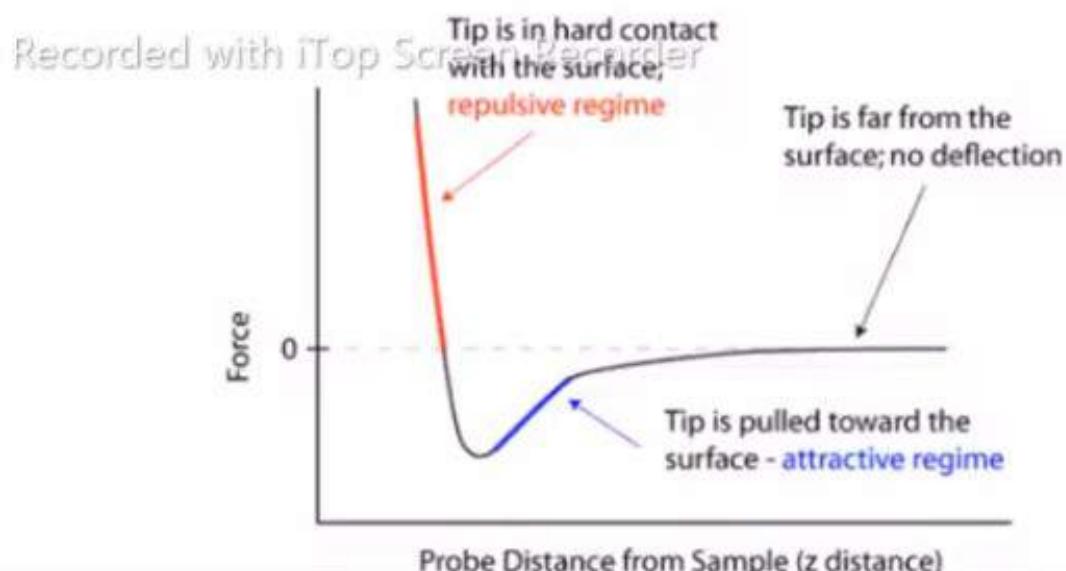
Measuring Forces in AFM

Because the Atomic Force Microscope relies on the forces between the tip and sample, these forces impact AFM imaging. The force is not measured directly, but calculated by measuring the deflection of the lever, knowing the stiffness of the cantilever.

Hooke's law gives:

$$F = -kz$$

where F is the force, k is the stiffness of the lever, and z is the distance the lever is bent.



Contact Modes for AFM

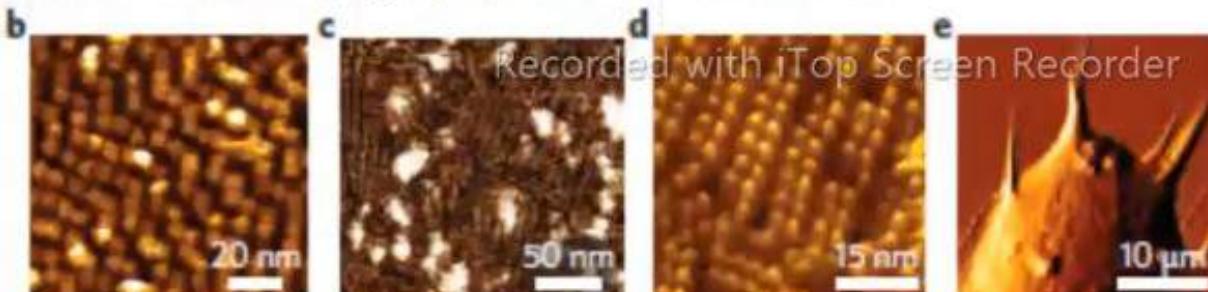
Widely used among all modes

Contact Mode

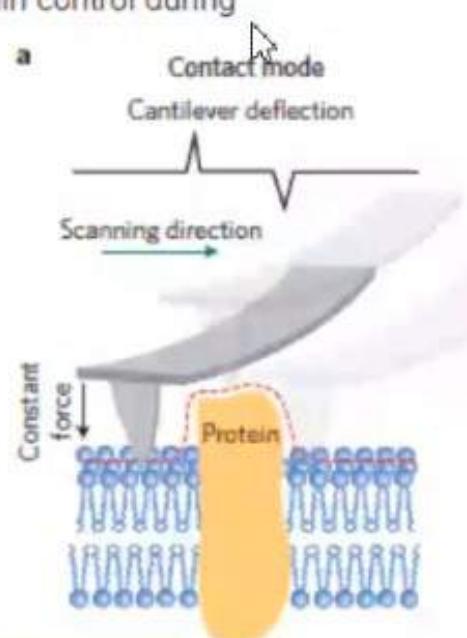
The first and original mode of operation is contact mode. As the tip is raster-scanned across the surface, the lever is deflected as it moves over the surface topography.

There are two methods of imaging in contact mode: constant force or constant height. In constant force mode, the tip is continually adjusted to maintain a specified deflection. It is this adjustment that is used to display an image.

When the tip scans in constant height mode, the sample must be relatively flat for the feedback loop to maintain control during scanning. This is useful for small, high-speed atomic resolution scans.



AFM topographs. **b**, Cyclic nucleotide-regulated potassium channels (MlotiK1) reconstituted into lipid membranes. **c,d**, Rows of densely packed rhodopsin dimers distributed in the native disc membrane extracted from rod outer segments of the eye. **e**, Image of a living SAOS-A2 cell bundling and pulling collagen fibrils coating a substrate. To maximize contrast, the exemplified image shows the deflection of the cantilever,

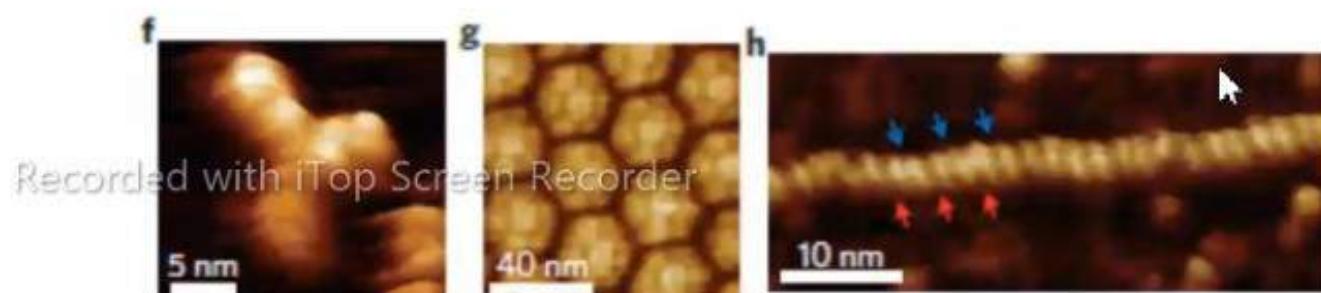
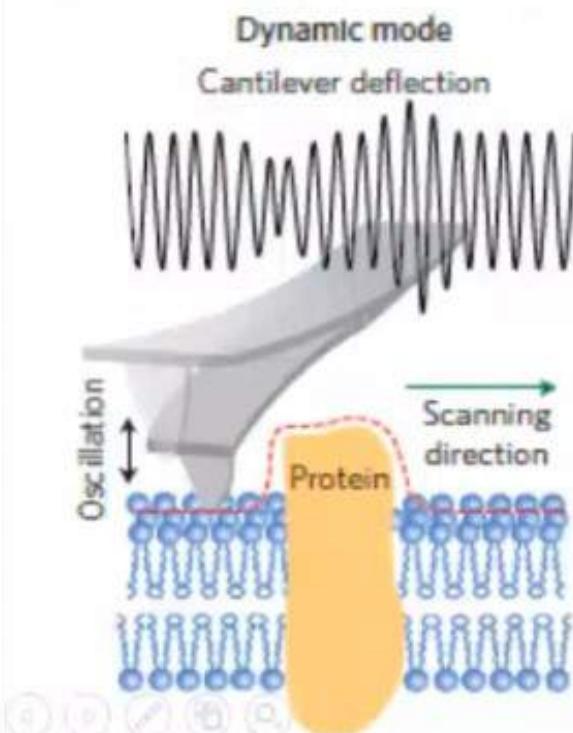


Dynamic Force Mode

Also known as... tapping mode, intermittent-contact mode, acoustic AC mode or oscillating mode.

A stiff cantilever is oscillated closer to the sample than in noncontact mode. Part of the oscillation extends into the repulsive regime, so the tip intermittently touches or taps the surface. Very stiff cantilevers are typically used, as tips can get stuck in the water contamination layer.

The advantage of tapping the surface is improved lateral resolution on soft samples. Lateral forces such as drag, common in contact mode, are virtually eliminated. For poorly adsorbed specimens on a substrate surface, the advantage is clearly seen.

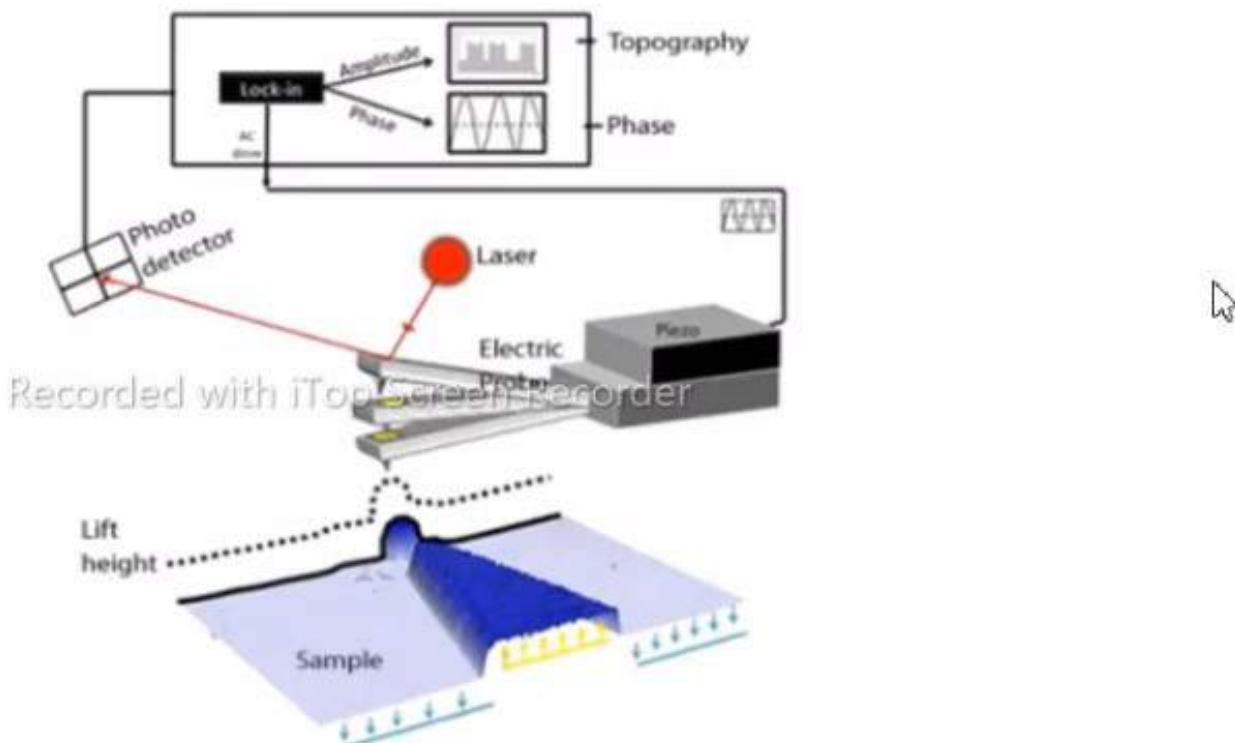


f-h, Dynamic mode AFM

topographs. f, An IgG antibody absorbed to mica and visualized with frequency modulation mode. g, Single bromelain mosaic viruses packed in a crystalline assembly. h, Circular plasmid DNA imaged in buffer solution

Electric Force Microscopy

EFM oscillates a conducting AFM tip to sense electric force gradients. Usually, this is done in 2 passes – one to measure the topography in a standard dynamic mode and the second to “lift” a set amount above the recorded profile to measure electric field strength. Using the phase signal, a map of the gradient of the electric field is created.



Schematic of EFM operation in lift mode with a second pass at a defined height above the surface to sense electric field strength

Table 1 | Comparison of high-resolution imaging techniques in molecular and cell biology.

Technique/feature	Atomic force microscopy	Super-resolution microscopy (STED, PALM, STORM)	Transmission electron microscopy	Scanning electron microscopy
Resolution	≤1 nm–50 nm*	20–50 nm	0.2–10 nm	2–10 nm
Sample preparation and environment	Sample on support; physiological (buffer solution, temperature, CO ₂)	Fluorescence labelling; physiological (buffer solution, temperature, CO ₂)	Sample on grid; dehydrated (negative stain); vitrified (cryo-electron microscopy)	Freeze/critical point drying and metal shadowing
Artefacts	Tip, force, scanning	Bleaching, toxicity	Dehydration, ice crystal formation, beam damage	Dehydration, metal shadowing, beam damage
Advantages	Imaging under native conditions; no staining labelling or fixation necessary; high signal-to-noise ratio; assessment of multiple physical, chemical and biological parameters	Access to three-dimensional cellular structures; high spatiotemporal resolution; monitoring biomolecular processes in live cells	Solves atomic structures of proteins; conformational snapshots of proteins and complexes; molecular- resolution structures within the cell	Imaging surfaces of tissues, cells and interfaces at nanometre-scale resolution
Limitations	Restricted to surfaces	Imaging restricted to fluorescence labels	No life processes	No life processes

Whole cell, extracted cellular or synthetic membranes, purified proteins and nucleic acids are considered. *On membrane proteins, resolution ≤1 nm can be achieved, on mammalian cells ~50 nm and on microbial cells ~10 nm. STED, stimulated emission depletion; PALM, photoactivated localization microscopy; STORM, stochastic optical reconstruction microscopy.

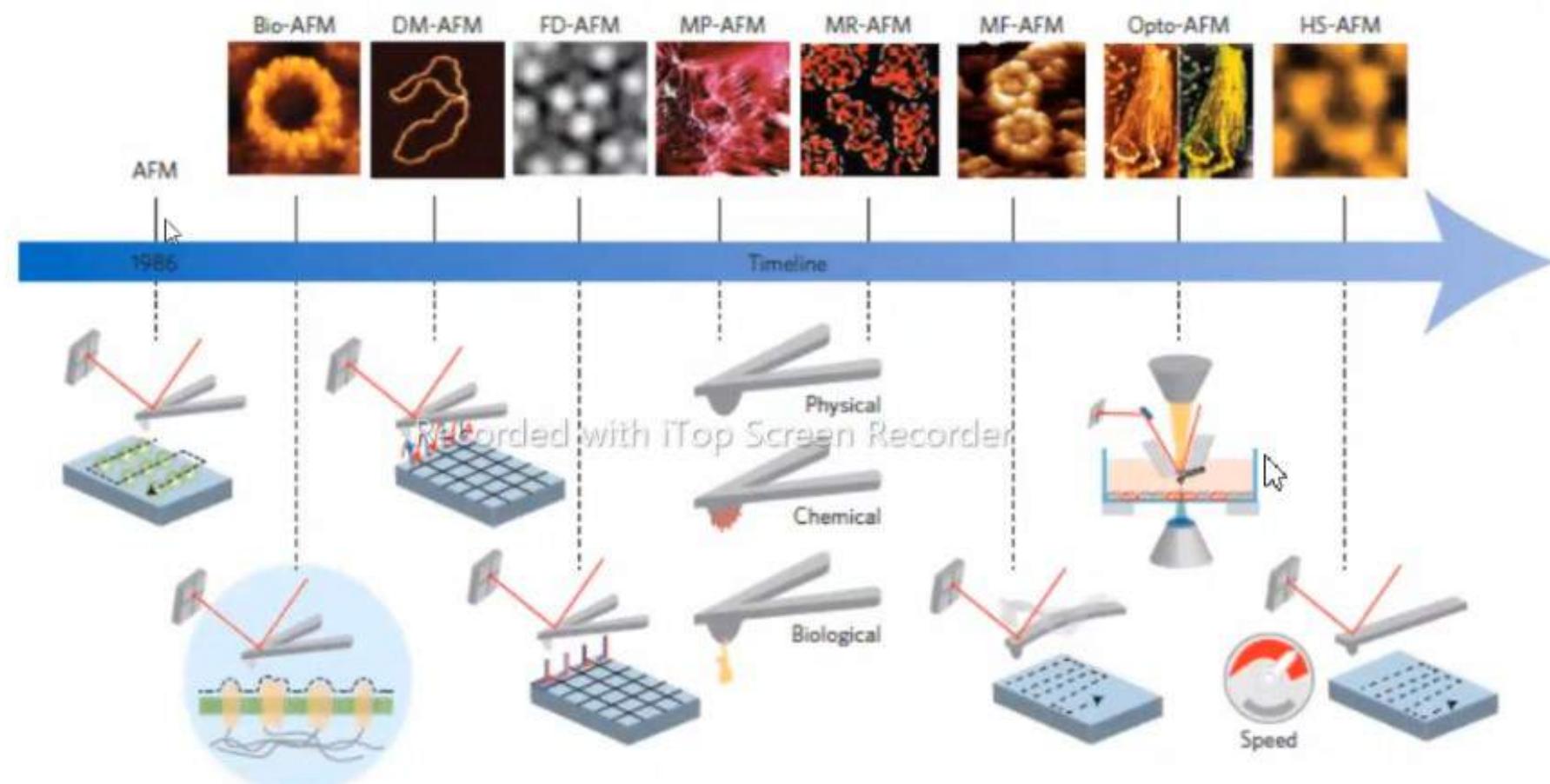


Figure 1 | Timeline of key inventions, starting from the birth of AFM in 1986 to the latest AFM imaging modes in molecular and cell biology. Key inventions

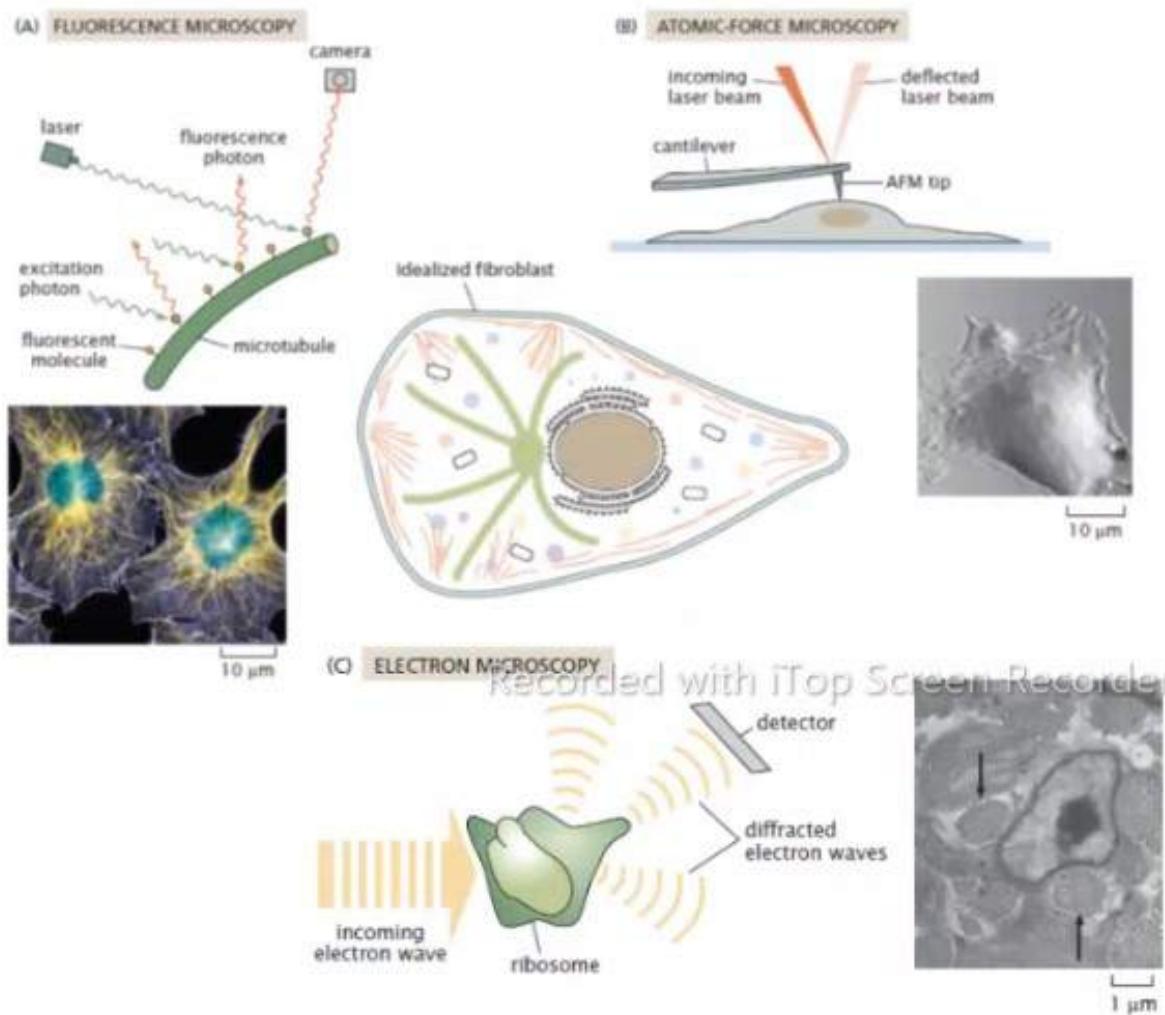


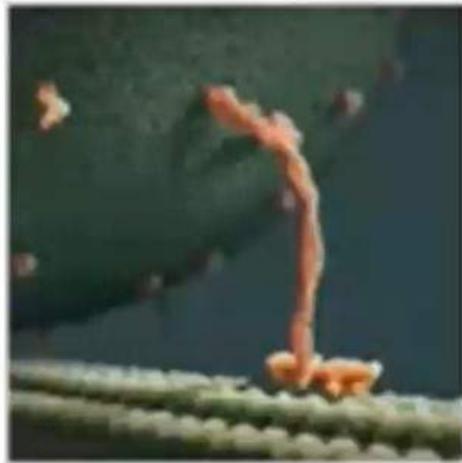
Figure 2.13: Experimental techniques that have revealed the structure of both cells and their organelles. (A) Fluorescence microscopy and the associated image of a fibroblast with labeled microtubules (yellow) and DNA (green). (B) Atomic-force microscopy schematic and the associated image of the surface topography of a fibroblast. (C) Electron microscopy schematic and image of cross-section through a fibroblast in an animal tissue. Arrows indicate bundles of collagen fibers. (A, courtesy of Torsten Wittman; B, adapted from M. Radmacher, *Meth. Cell Biol.* 83:347, 2007; C, adapted from D. E. Birk and R. L. Trelstad, *J. Cell Biol.* 103:231, 1986.)

Biophysical Methods



REFRESHER ON PROTEINS

Proteins Supervise everything in body



Muscles contract because
proteins crawl on top of
each other

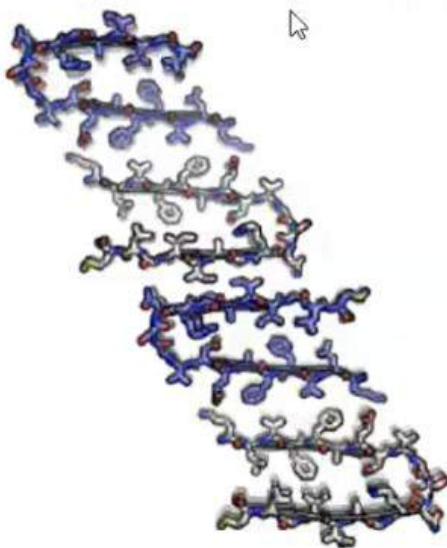


Proteins build up Structure

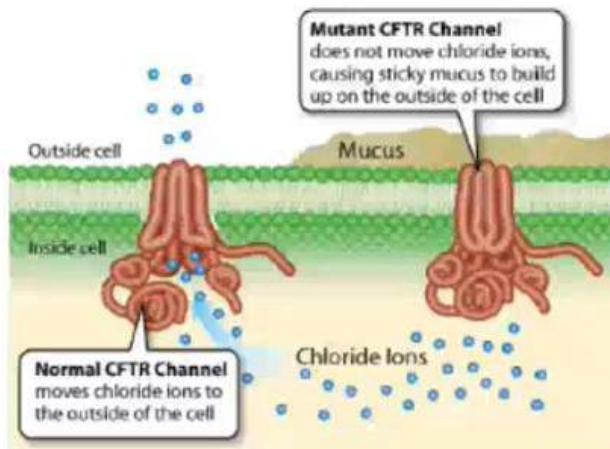
REFRESHER ON PROTEINS

Proteins are machines of Life

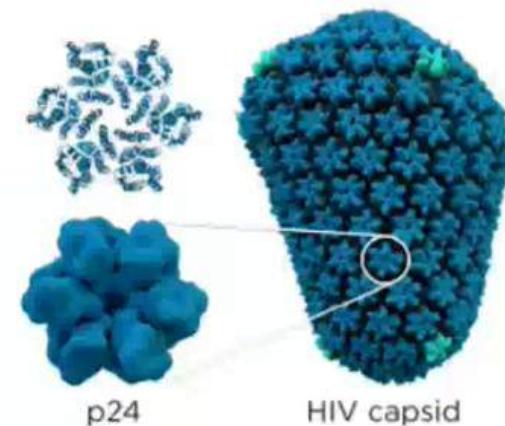
Everything that happens in our body happens because a protein supervises it, even when things go wrong



Protein aggregates cause Alzheimer's disease



A single “unlucky” mutation causes cystic fibrosis



Bacteria and virus need proteins to function

REFRESHER ON PROTEINS



- 20 types of amino acids form various proteins
- Each amino acid consists of NH_2 , COOH and side group (R)
 - Every **amino acid can be hydrophobic or hydrophilic.**
- Proteins formed due to condensation process of amino acids resulting in a CO-NH (Peptide bond)

Conformation

- Active Form of Protein
- Folded 3D structure of Protein

Denatured

- Unfolded structure
- Inactive Form

REFRESHER ON PROTEINS

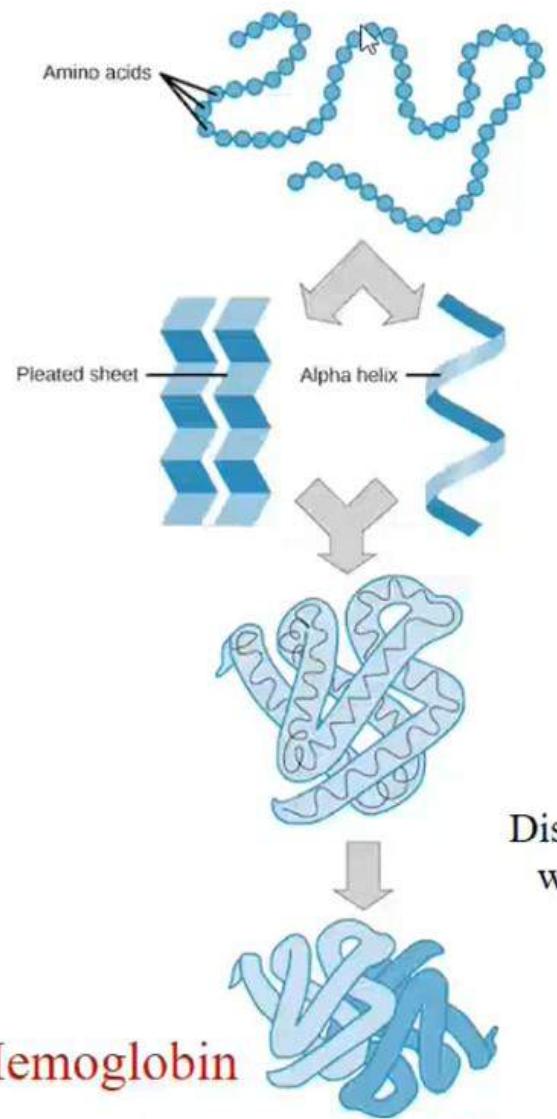
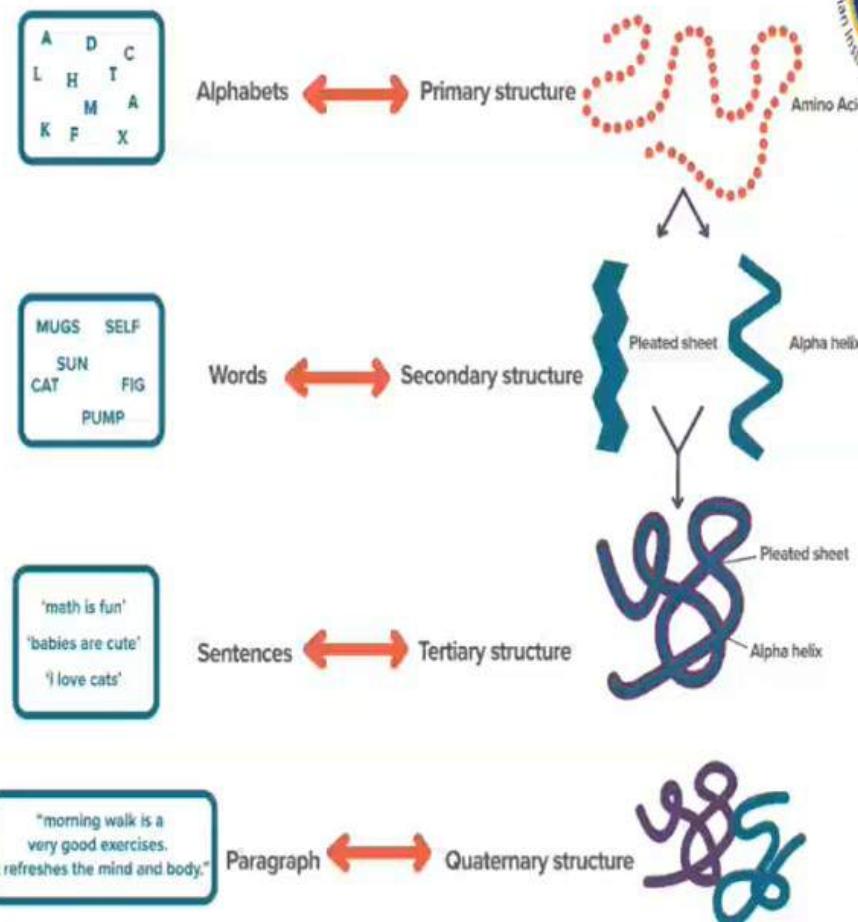


Figure from Rob Phillips Physical Cell Biology



A	D	C
L	H	T
M	M	A
K	F	X

MUGS	SELF
SUN	
CAT	FIG
PUMP	

'math is fun'
'babies are cute'
'I love cats.'

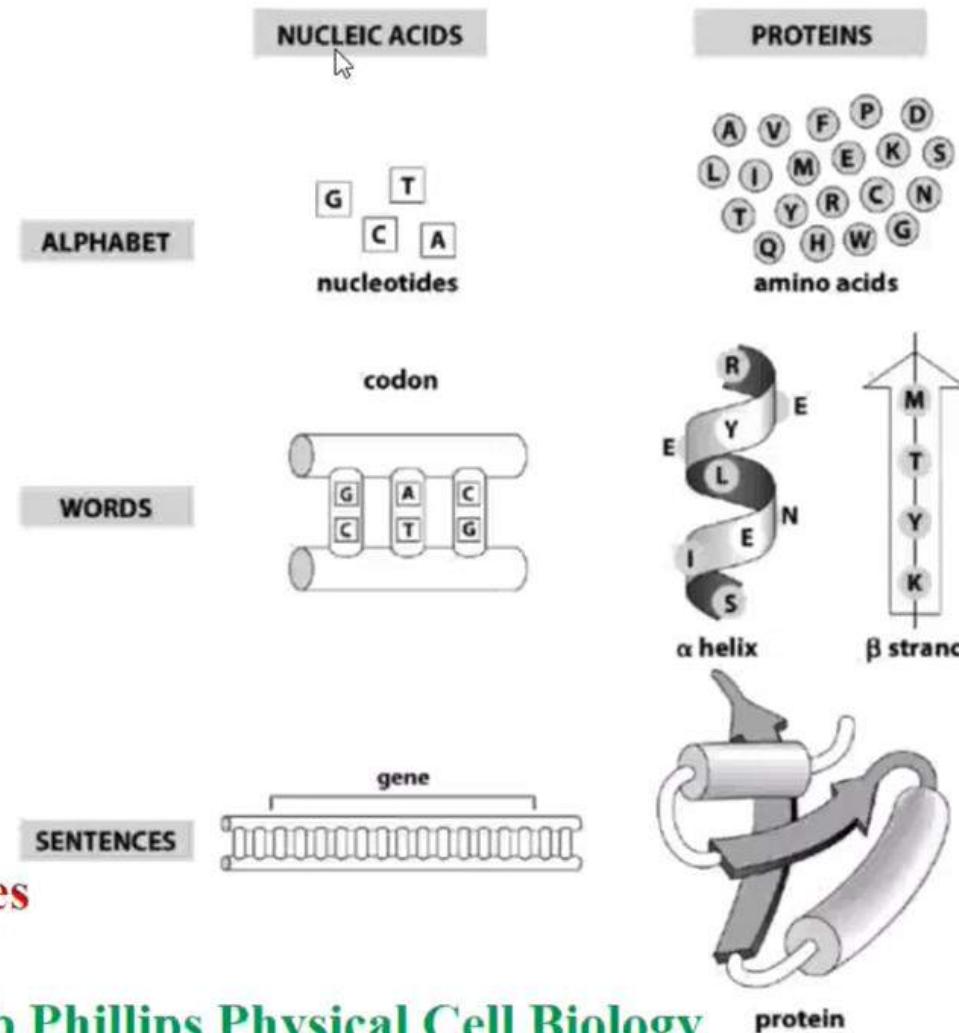
"morning walk is a very good exercise. It refreshes the mind and body."
--

Protein Structures

Polymer languages of Macromolecules

Polymer Nature of Macromolecules:

- (1) DNA and RNA molecules: made of nucleic acids
- (2) proteins: made of amino acids



Combination of three nucleotides encode for one amino acid

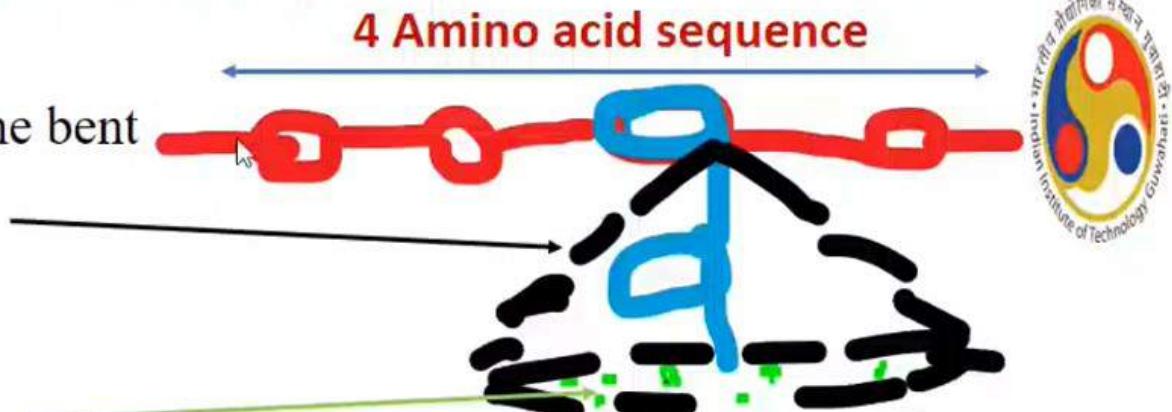
Figure from Rob Phillips Physical Cell Biology



Why is determining Protein Folding a difficult problem??

- Folded structure of sequence determined by sequence of successive solid bend angles.

Cone is the range around which the bent sequence can wriggle



- We can limit the chain orientations in cone (say discretize to seven solid angles in cone(In green)).
- Hence determining the shape is the greatest computational problem.

Content from Prof. Steven Skiena lectures and Rob Philips (Physical Cell Biology)



Physics works, but the phase space of a protein is huge!

You need to see many of these



before seeing this

We need to reduce the search space!

Slide for Dr.Emiliano Brini Talk on MELD method for protein folding prediction

Protein Folding

insight review articles

Protein folding and misfolding

Christopher M. Dobson

University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK (e-mail: cmd44@cam.ac.uk)

The manner in which a newly synthesized chain of amino acids transforms itself into a perfectly folded protein depends both on the intrinsic properties of the amino-acid sequence and on multiple contributing influences from the crowded cellular milieu.

Depends On- Free Energy

Protein folding and misfolding

Christopher M. Dobson

University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK (e-mail: cmd44@cam.ac.uk)



The fundamental mechanism of protein folding

The concept of an energy landscape

The mechanism by which a polypeptide chain folds to a specific three-dimensional protein structure has until recently been shrouded in mystery. Native states of proteins almost always correspond to the structures that are most thermodynamically stable under physiological conditions³. Nevertheless, the total number of possible conformations of any polypeptide chain is so large that a systematic search for this particular structure would take an astronomical length of time. However, it is now clear that the folding process does not involve a series of mandatory steps between specific partly folded states, but rather a stochastic search of the many conformations accessible to a polypeptide chain^{3–5}.

HP Model for Amino Acids

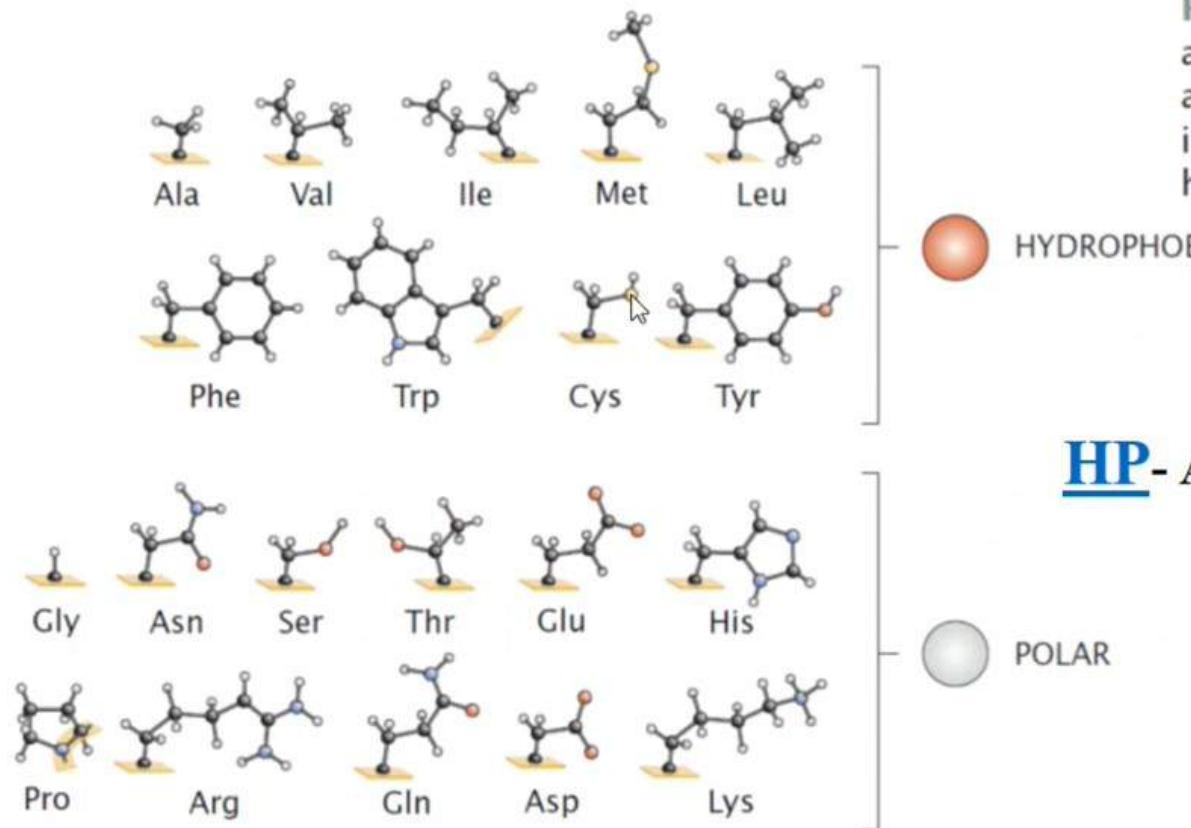
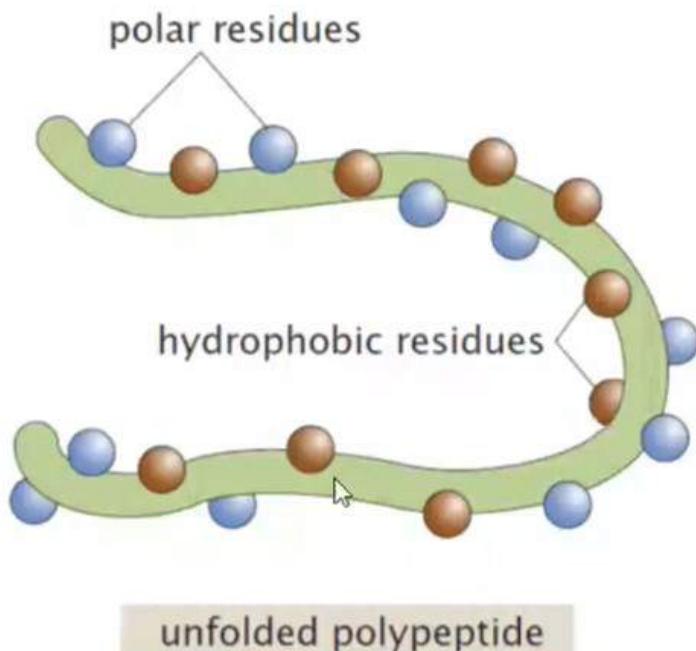


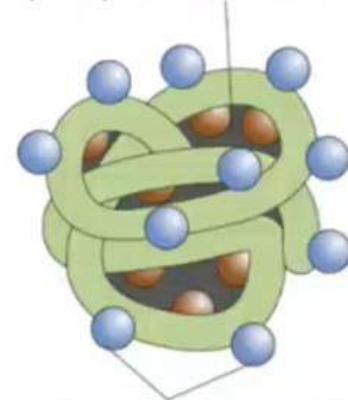
Figure 8.28: Mapping of the amino acids onto an HP alphabet. The 20 amino acids are coarsely separated into two categories, namely, hydrophobic (H) or polar (P).

HP- Amino acids classified based on similar properties

Schematic of Protein Folding

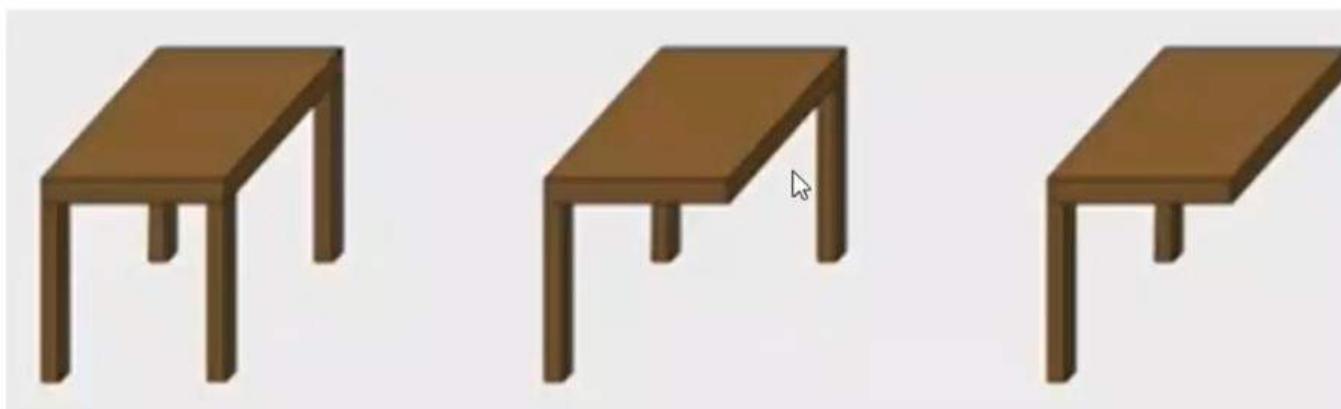


free energy lowered by sequestering hydrophobic residues



polar residues participate in hydrogen bond network

folded conformation in aqueous environment



Protein Shape Importance determines function

Proteins are long molecules, their shape is determined by the interactions between their components

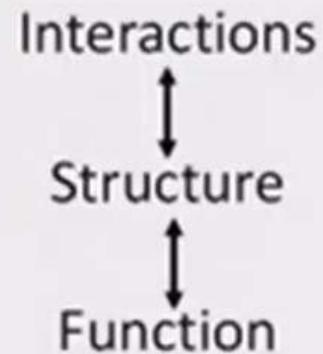
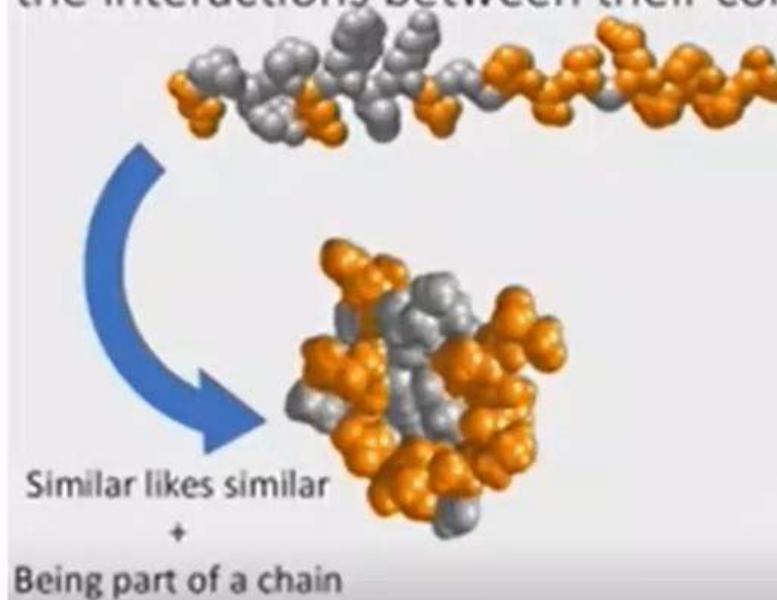
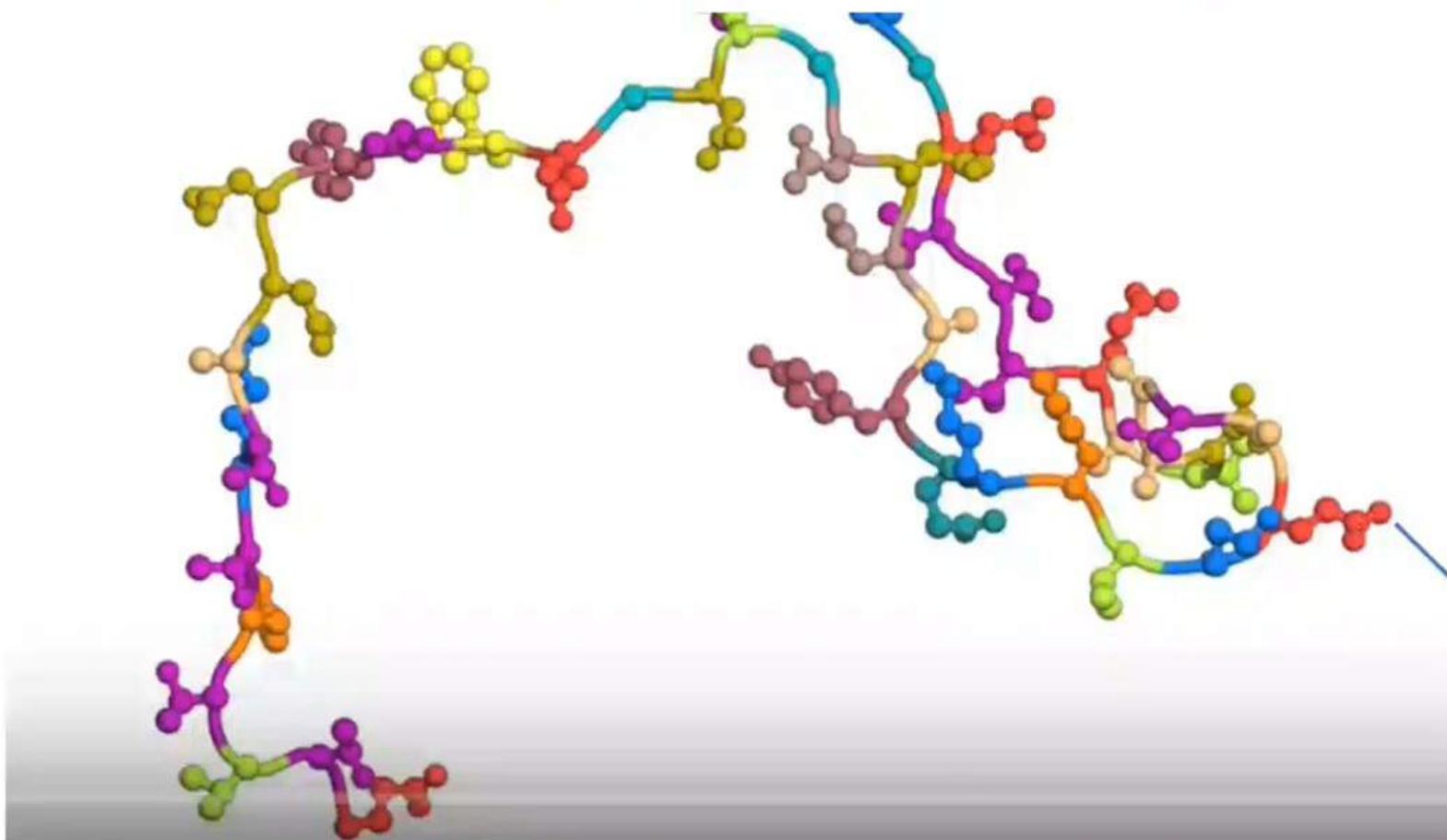


Figure from Rob Phillips
Physical Cell Biology

Example:1 Protein Molecule Visualized (Long and stringy)



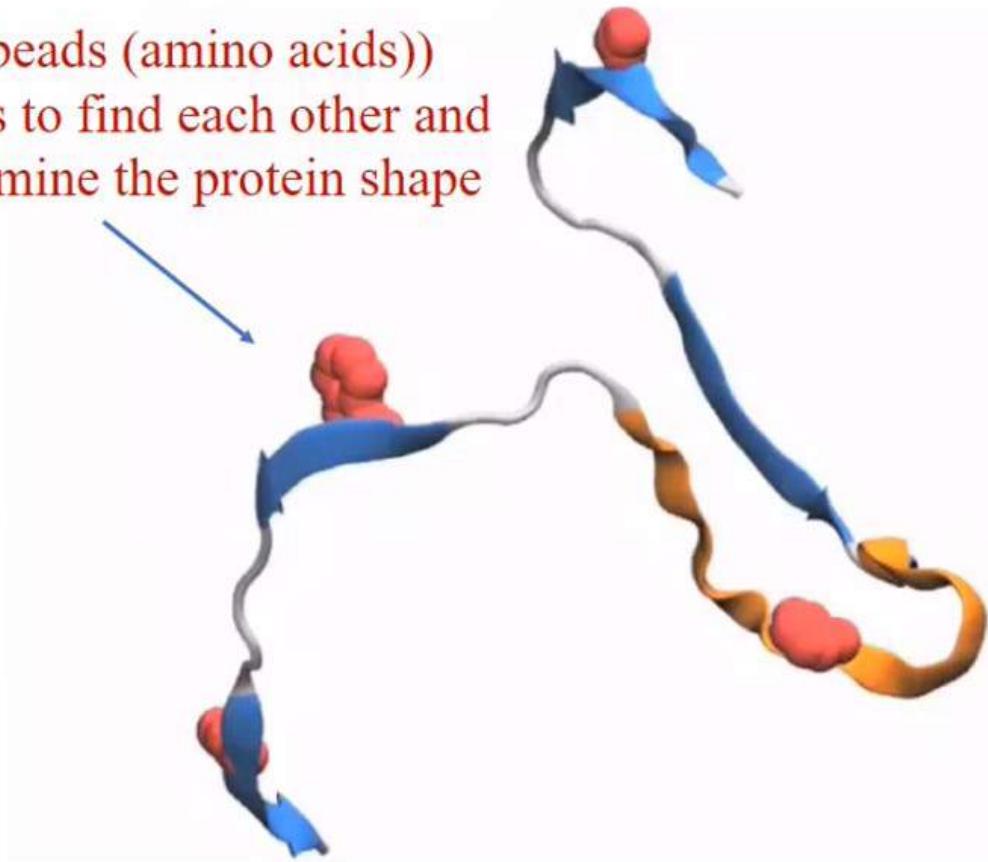
False colors
indicate the
various amino
acids

Lines are bonds

Protein Structure can be viewed on a)NMR b)X-crystallography



Red beads (amino acids)
needs to find each other and
determine the protein shape



Protein shape determines protein function

Content from Prof. Ken Dill TED talk, State University of New York at Stony Brook

The nature of protein folding pathways

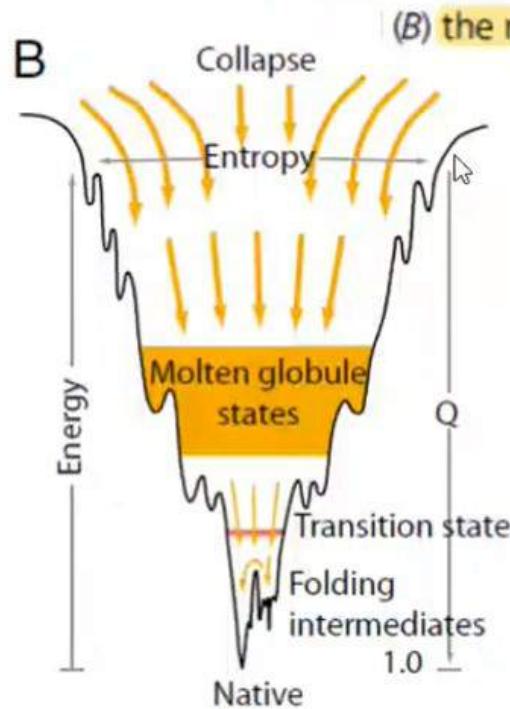
S. Walter Englander¹ and Leland Mayne

Johnson Research Foundation, Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Edited by Alan R. Fersht, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom, and approved September 23, 2014 (received for review June 24, 2014)



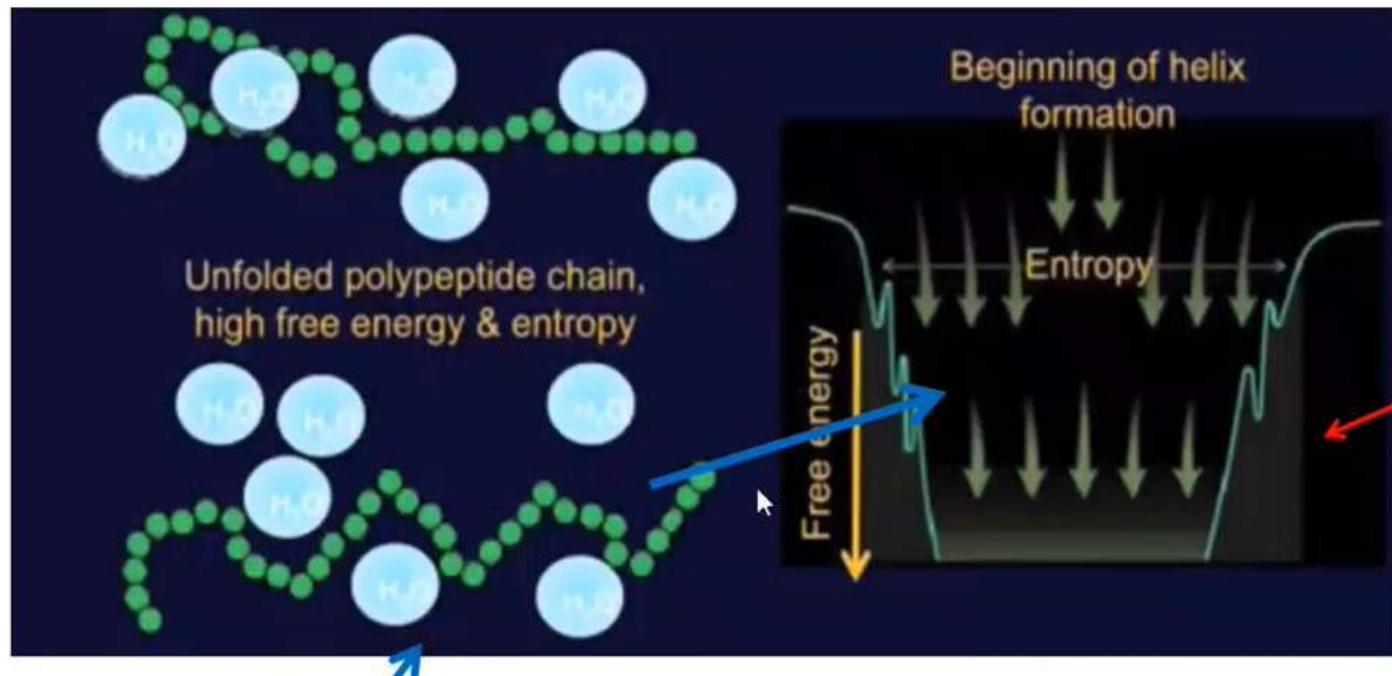
Viewing Protein Folding Through a Funnel



How Does a Protein Reach its Ordered Folded State from its Unfolded Ensemble?

What is the nature of the ordering in a folded native protein, and how does that ordering arise from its highly disordered denatured state? The basic ideas are expressed through statistical mechanics. The relative stabilities of states depend on their free energies. At equilibrium, the probability of occupying a state depends on its Boltzmann's weight, $\exp(-\Delta G/k_B T)$, where ΔG is the difference in free energies of the states, native and unfolded in this case, k_B is Boltzmann's constant and T is temperature.^{48,49} Small proteins typically fold cooperatively, i.e. through relatively sharp transitions between the disordered and ordered states.^{50,51}

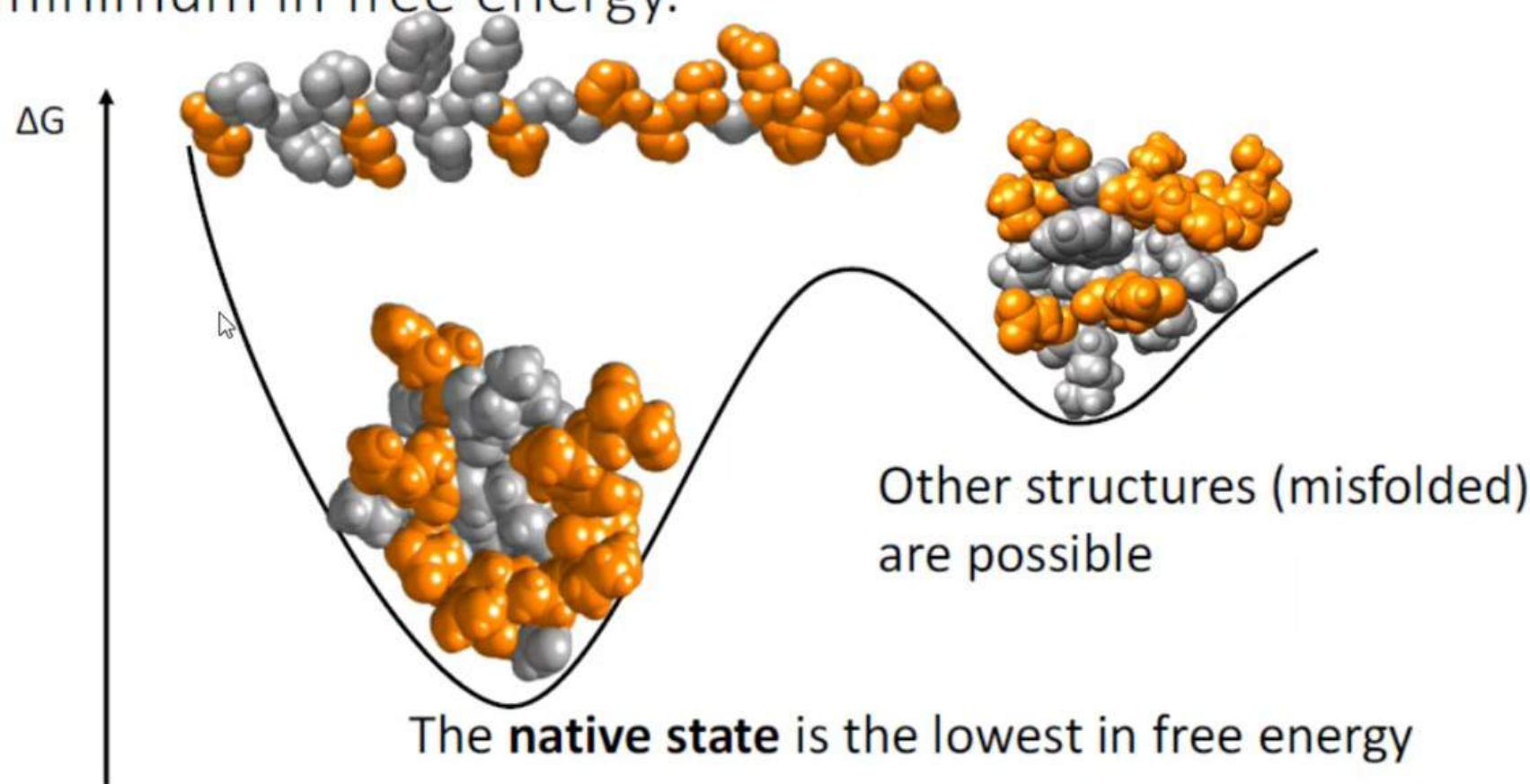
Middle of Funnel (During Protein Folding)



Entropy of the amino acid chain decreases in Middle of funnel

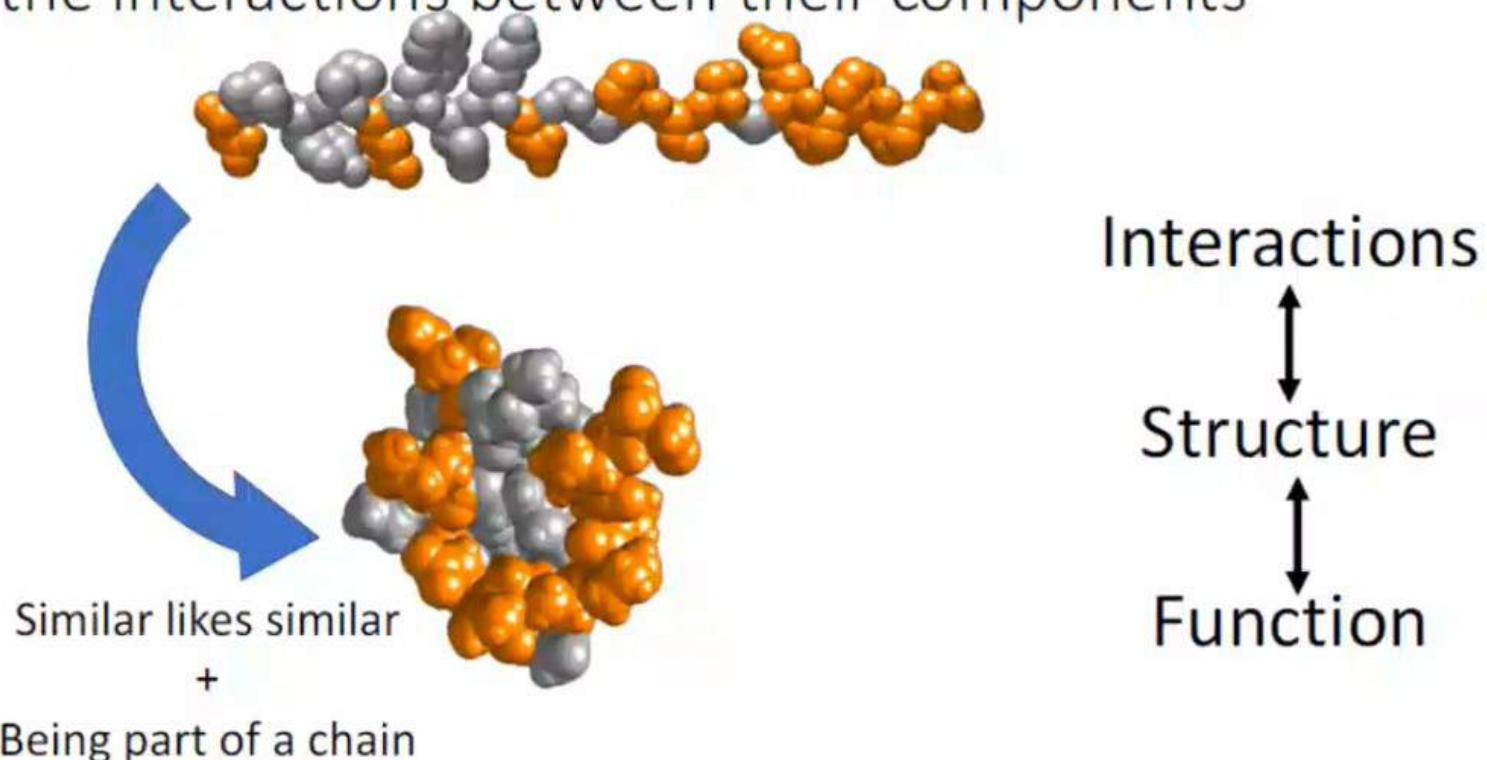
Entropy of surrounding water molecules increases down the funnel

Physics teaches us that the most stable structure is the minimum in free energy.



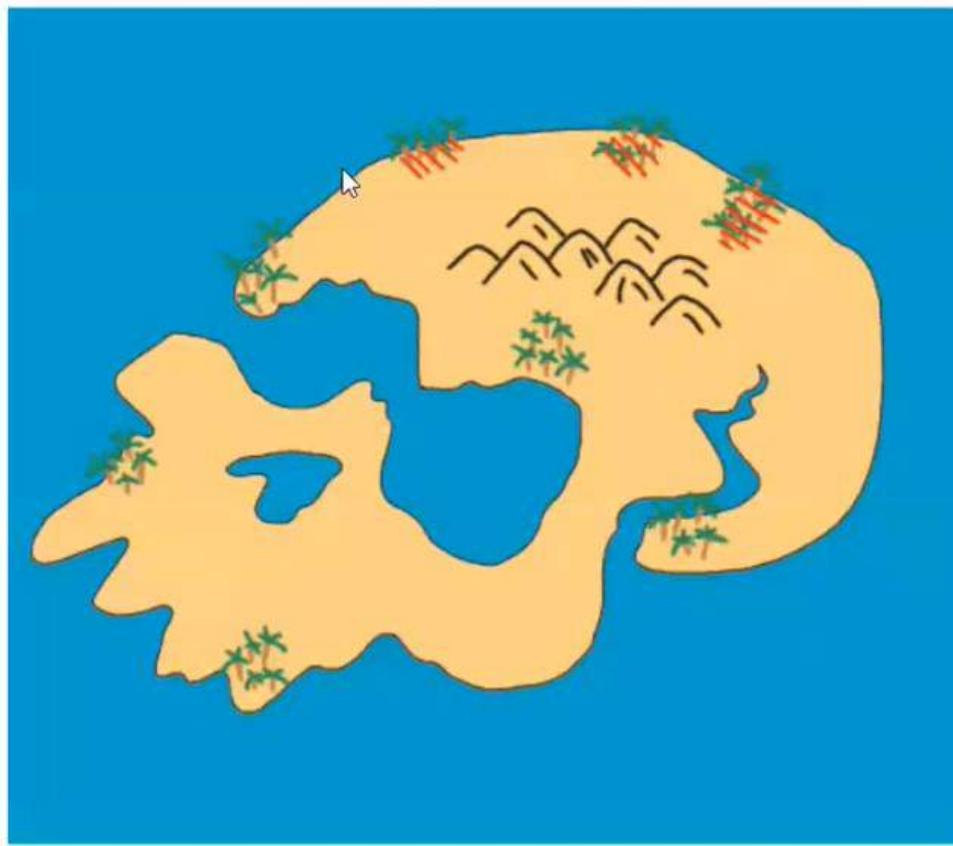
Slide for Dr.Emiliano Brini Talk on MELD method for protein folding prediction

Proteins are long molecules, their shape is determined by the interactions between their components



Slide for Dr.Emiliano Brini Talk on MELD method for protein folding prediction

Knowledge helps reducing the search space



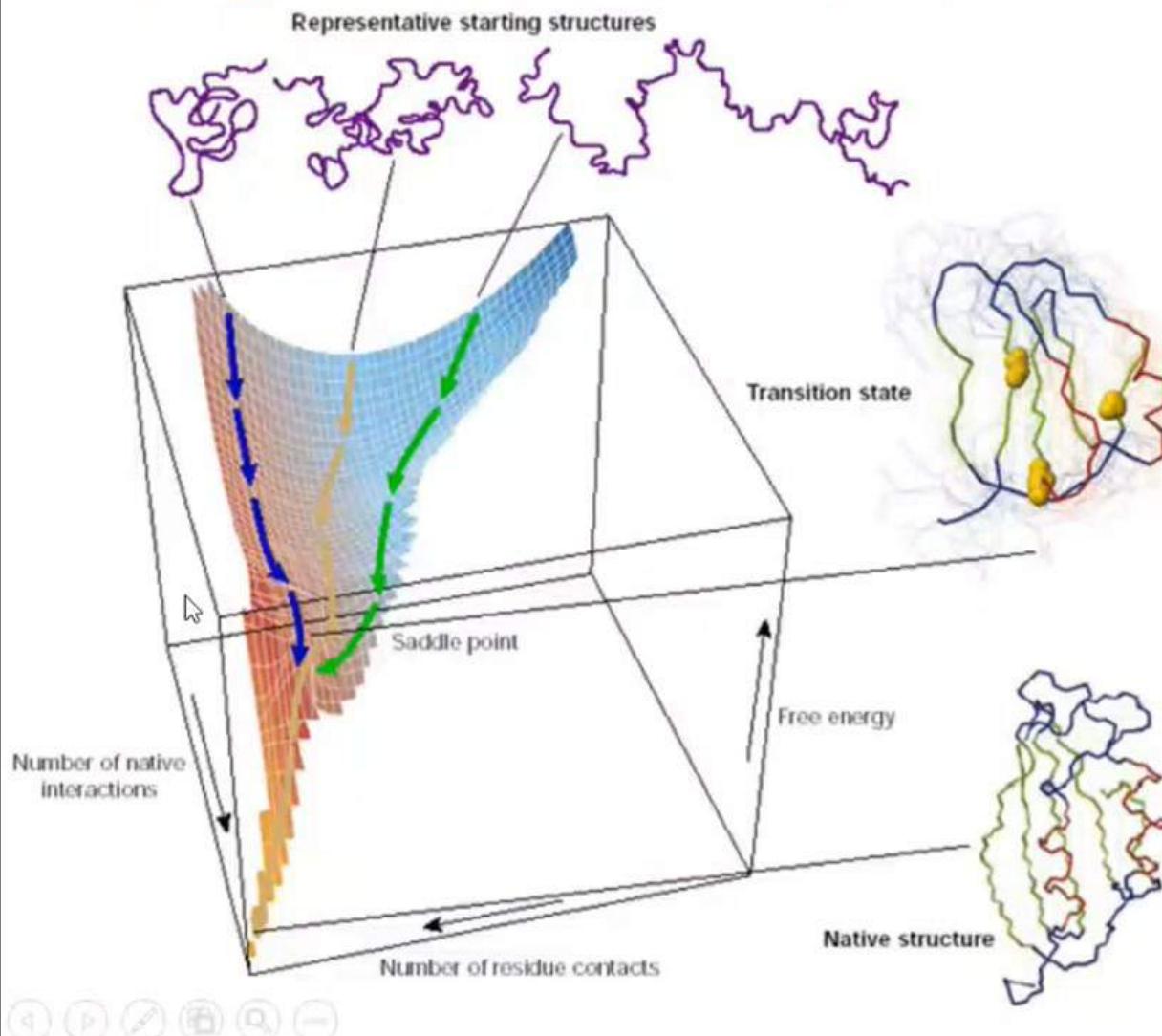
The treasure is:

- in a palm forest
- North of the mountains

Physics is a really efficient metal detector, but it needs to be guided

Slide for Dr.Emiliano Brini Talk on MELD method for protein folding prediction

Energetic Funnel (more parameters) during Protein folding



- Proteins find its functional state in microseconds.
- **Genetic algorithms, simulated annealing concepts** in computer science used to find the global minimum

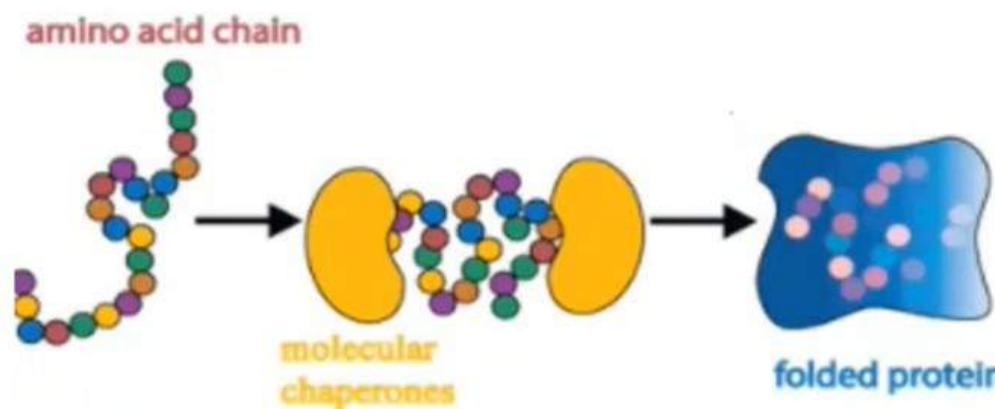
Dinner, A. R., *et al.* Understanding protein folding via free energy surfaces from theory and experiment. *Trends in Biochemical Sciences* **25**, 331–339, 2000.

Chaperones for Protein Folding

Class of heat inducible protein which provide kinetic assistance in folding

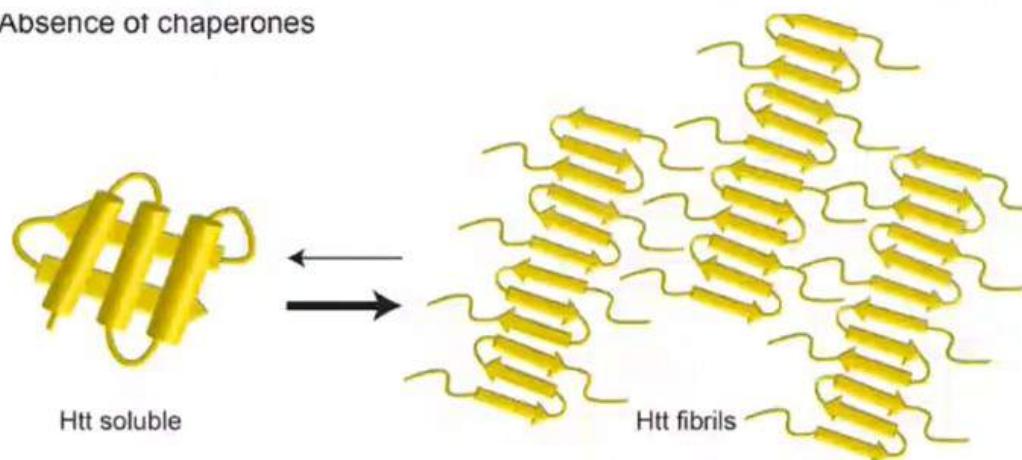
(Heat shock proteins-An example chaperone) help correctly fold by binding to their hydrophobic surfaces

Chaperones are proteins with abilities of helping other proteins to mature, to avoid improper contacts and to correct errors

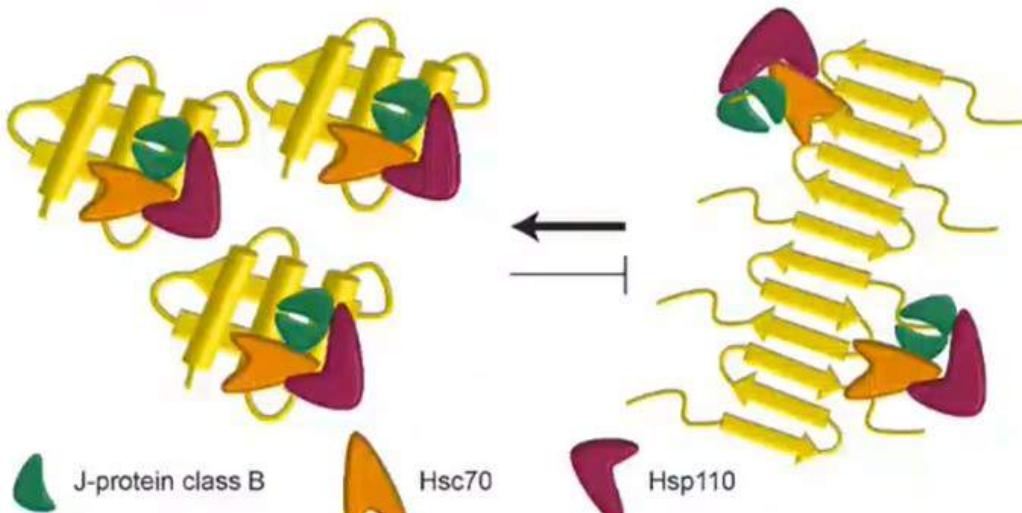


Chaperones for Protein Folding (Huntington disease)

Absence of chaperones



Presence of chaperones



Complete suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex

Annika Scior, Alexander Buntru, Kristin Arnsburg, Anne Ast, Manuel Iburg, Katrin Juenemann, Maria Lucia Pigazzini, Barbara Mlody, Dmytro Puchkov, Josef Priller, Erich E Wanker, Alessandro Prigione, Janine Kirstein

Author Affiliations

DOI 10.15252/embj.201797212 | Published online 06.12.2017
The EMBO Journal (2018) 37, 282-299

Chaperones suppressed the build-up of pathogenic amyloid (Htt) fibrils and disaggregates them

Huntington's is an inherited disease that causes the progressive breakdown (degeneration) of nerve cells in brain

(Pioneers/Active Researchers) in Protein Folding



Ken A. Dill

Director of Laufer Center, Stony Brook University

Verified email at laufercenter.org

Statistical physics of protei...

FOLLOW

Cited by

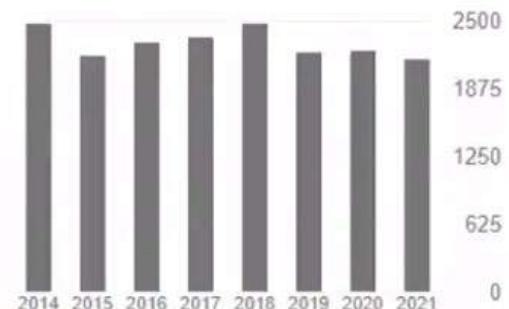
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TITLE

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YEAR

CryoFold: Determining protein structures and data-guided ensembles from cryo-EM density maps
M Shekhar, G Terashi, C Gupta, D Sarkar, G Debussche, NJ Sisco, ...
Matter

2021

MELD-accelerated molecular dynamics help determine amyloid fibril structures
B Sharma, KA Dill
Communications biology 4 (1), 1-11

2021

Nanoscale Catalyst Chemotaxis Can Drive the Assembly of Functional Pathways
C Kocher, L Agozzino, K Dill
The Journal of Physical Chemistry B 125 (31), 8781-8786

2021

The protein folding problem: The role of theory
R Nassar, GL Dignon, RM Razban, KA Dill
Journal of Molecular Biology, 167126

3 2021

Public access

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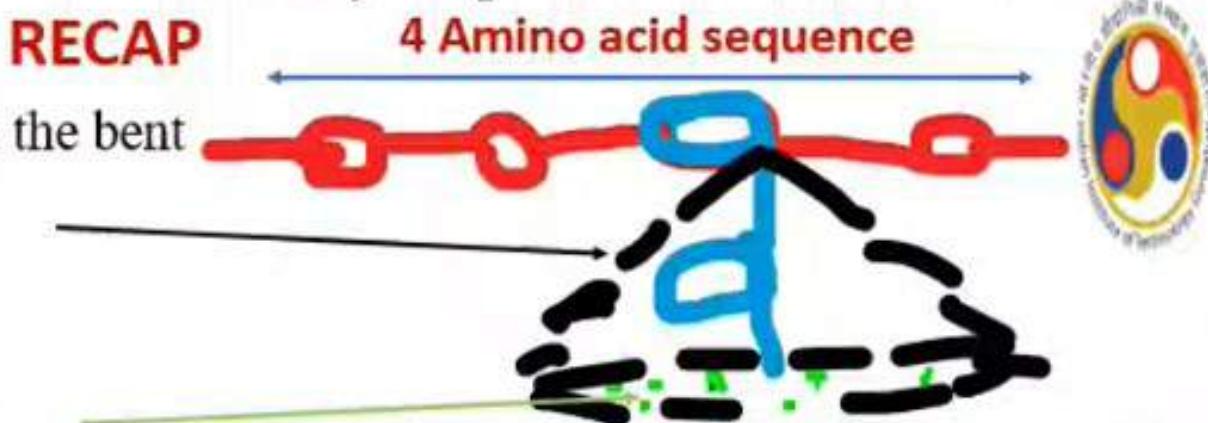
4 articles

107 articles

Why is determining Protein Folding a difficult problem??

- Folded structure of sequence determined by sequence of successive solid bend angles.

Cone is the range around which the bent sequence can wriggle



- We can limit the chain orientations in cone (say discretize to seven solid angles in cone(In green)).
- Hence determining the shape is the greatest computational problem.

Content from Prof. Steven Skiena lectures and Rob Philips (Physical Cell Biology)



Schematic of Protein Folding

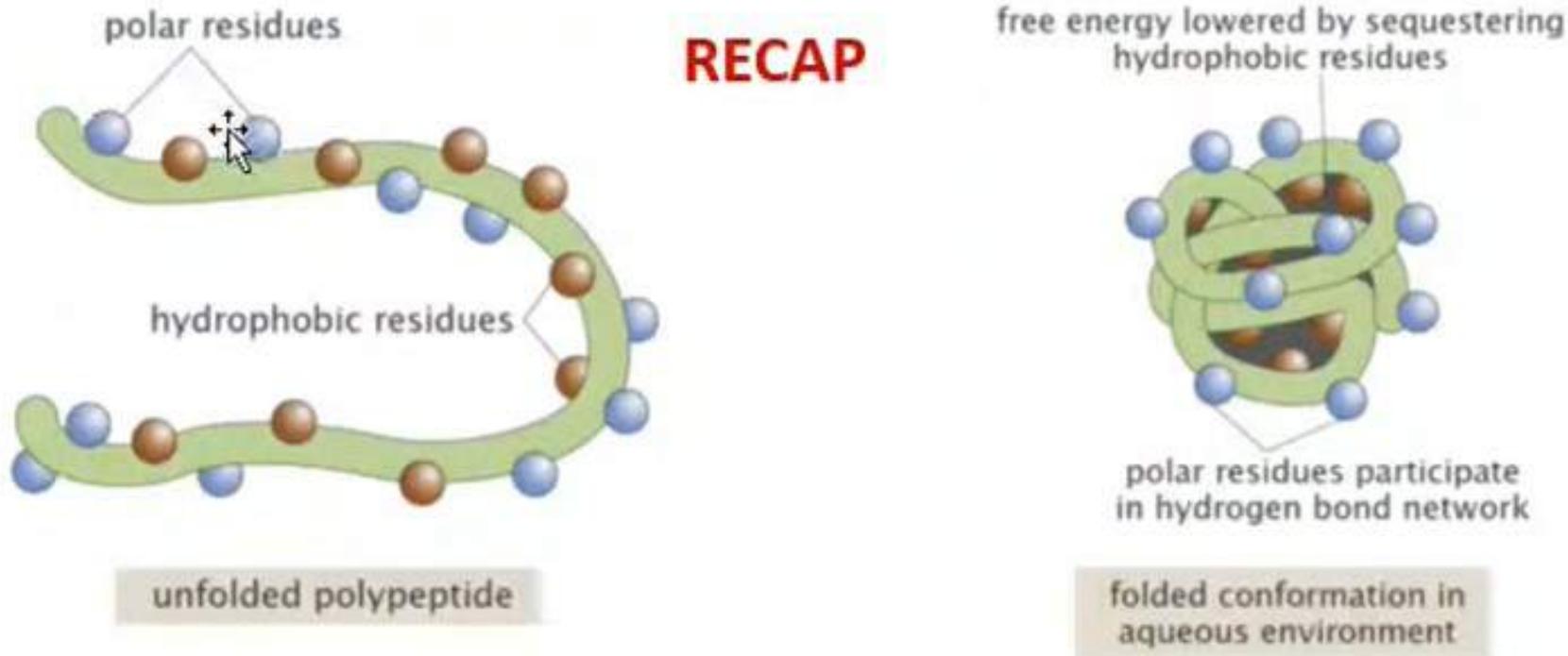
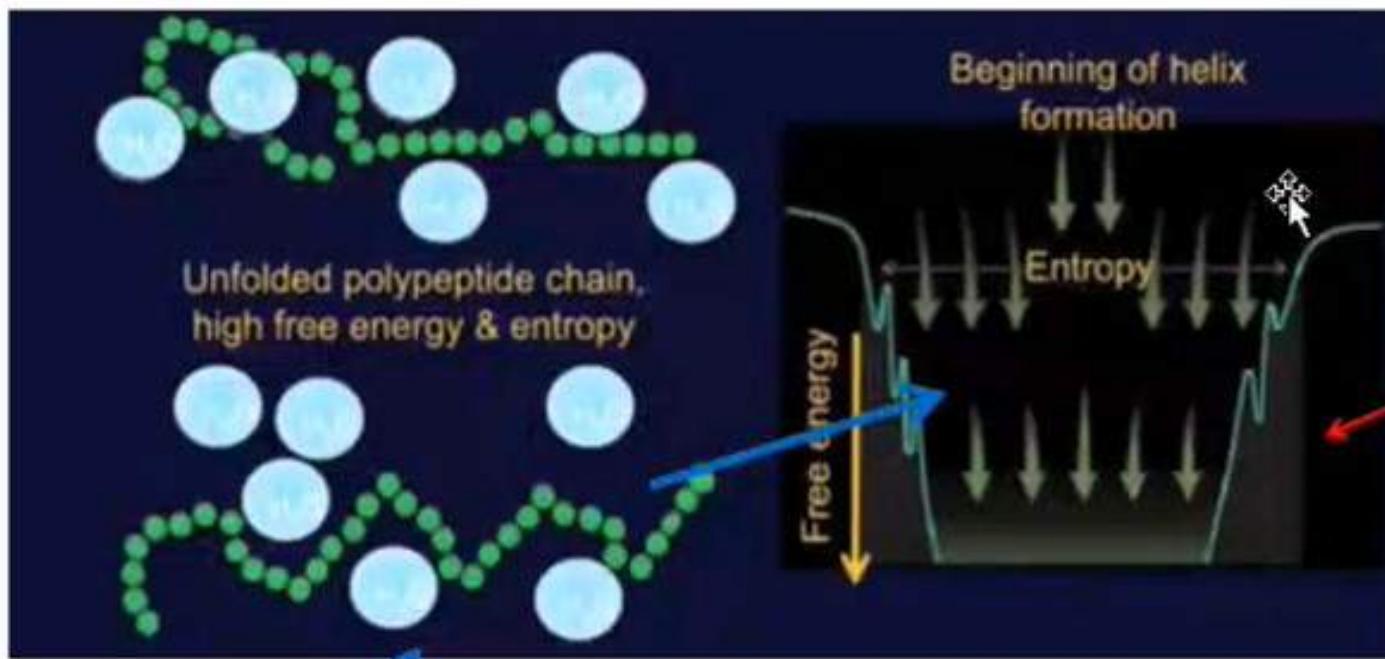


Figure from Rob Phillips Physical Cell Biology



Middle of Funnel (During Protein Folding)

RECAP



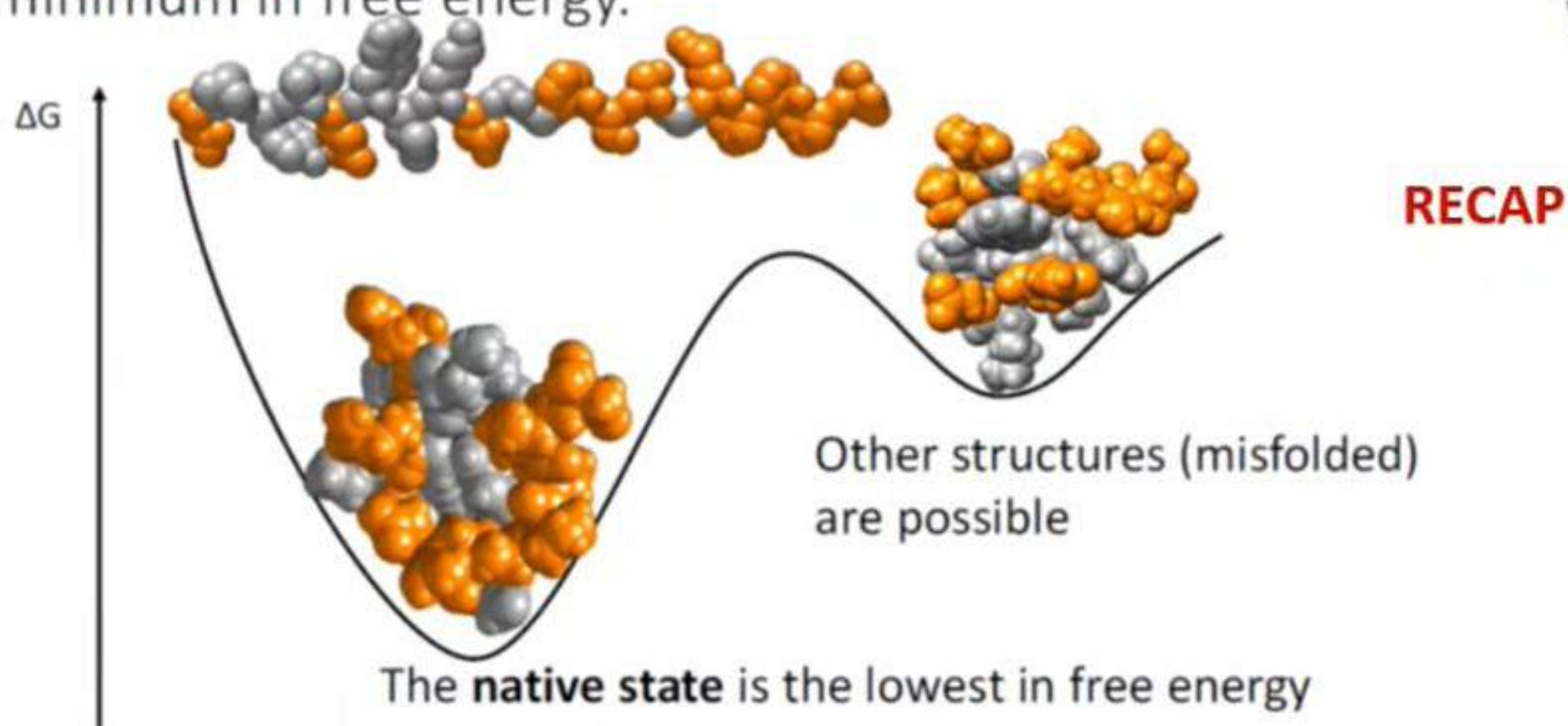
Hydrogen bonds interact with polypeptide chain ← Middle of the Funnel

Entropy of the amino acid chain decreases in Middle of funnel

Entropy of surrounding water molecules increases down the funnel



Physics teaches us that the most stable structure is the minimum in free energy.



Slide for Dr.Emiliano Brini Talk on MELD method for protein folding prediction

Food for THOUGHT

DeepMind's new protein-folding A.I. is already helping in the fight against COVID-19

fortune.com/2020/11/30/covid-protein-folding-deepmind-ai/

BY JEREMY KAHN

December 1, 2020 2:00 AM GMT+5:30

Interested can Investigate and discuss with me
(On email first)what is it about and if I find it true and
motivating, then the student can present to class



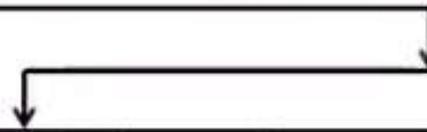
PROTEIN FOLDING/AGGREGATION



a. MATLAB APPS FOR PROTEIN ANALYSIS
(BIOINFORMATICS TOOLBOX)

b. PROTEIN MISFOLDING/AGGREGATION

c. DISORDERS DUE TO AGGREGATION



- Concept of Aggregation
- Energy Funnel (Aggregation Vs Folded proteins)



Acknowledgment: Aggregated Lecture from lot of resources
(Textbook/Journal Papers/Youtube lectures)

MATLAB Apps for PROTEIN ANALYSIS

(Make sure you Install Bioinformatics Toolbox from Mathworks)

Explore a Protein Sequence Using the Sequence Viewer App

R2021b



Overview Sequence Viewer

The Sequence Viewer app integrates many of the sequence functions in the Bioinformatics Toolbox™ toolbox. Instead of entering commands in the MATLAB® Command Window, you can select and enter options using the app.

Viewing Amino Acid Sequence Statistics

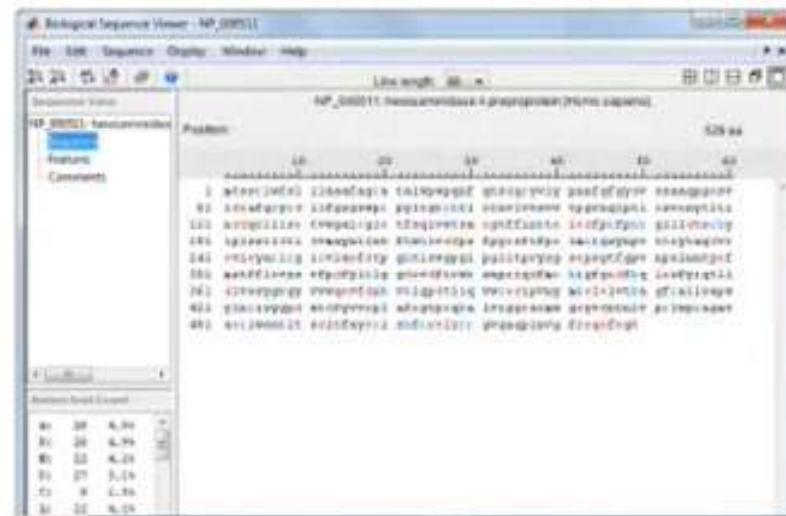
The following procedure illustrates how to view an amino acid sequence for an ORF located in a nucleotide sequence. You can import your own amino acid sequence, or you can get a protein sequence from the GenBank® database. This example uses the GenBank accession number NP_000511, which is the alpha subunit for a human enzyme associated with Tay-Sachs disease.

1. Select File > Download Sequence from > NCBI.

The Download Sequence from NCBI dialog box opens.

2. In the dialog box, type an accession number for an NCBI database entry, for example, NP_000511. Click the Protein option button, and then click OK.

The Sequence Viewer accesses the NCBI database on the Web and loads amino acid sequence information for the accession number you entered.



MATLAB Apps for PROTEIN ANALYSIS

(Make sure you Install Bioinformatics Toolbox from Mathworks)



3. Select Display > Amino Acid Color Scheme, and then select Charge, Function, Hydrophobicity, Structure, or Taylor. For example, select Function.

The display colors change to highlight charge information about the amino acid residues. The following table shows color legends for the amino acid color schemes.

Biological Sequence Viewer - NP_000511

File Edit Sequence Display Window Help

Line length: 60

NP_000511: hexosaminidase A preprotein [Homo sapiens]

Position: 529 aa

Amino Acid Color Legend:

Amino Acid	Color
A	Green
R	Red
N	Blue
D	Yellow
C	Orange
E	Purple
G	Light Green
H	Dark Blue
I	Light Orange
K	Dark Red
M	Light Blue
P	Light Green
S	Light Purple
T	Light Yellow
W	Light Orange
Y	Light Purple

Sequence View:

NP_000511: hexosaminidase A preprotein [Homo sapiens]

Position: 529 aa

Line length: 60

Sequence:

1 atsarlufsl liasafaga talppupqnf qudqryvly pmfqfydv maappgcev
61 ldeafqtyxd lfgggswpr pylqkrbt lunvlvvvv upgcuqlptl rzvenytiti
121 uddqulllse tvvgallle tfuqlvwksa egfffinkte iedfpifphr qllldtoryh
181 lplsuuldl dvmuynkinv fhuhlvddpx fpyesffpe lmrkgeyhpv thytcqdvk
241 evieyarlrg icvlaefdtp ghelwppgi polltpcysd sepsatfggv npslmntyef
301 matfflevss vfpdfylhlg udevdftcwk snpeiqdfml khgfgedfhq lesfyiqtll
361 divasygkg vuuqcvfdnk vhqpdtiiq vuuqedipvny uklelvka gfralisapv
421 ylinrisyqpd ukdfyvvepl uafgtpegka lviggeacav ugryvdutlv prlwpragav
481 acclwunkuit sultfayecl shfucellltt gvqugqpunvd fceqefeqt

Features:

Comments:

Amino Acid Count:

Amino Acid	Count	Percentage
A	26	4.9%
R	26	4.9%
N	22	4.2%
D	27	5.1%



MATLAB Links on PROTEIN ANALYSIS

<https://in.mathworks.com/help/bioinfo/ug/visualizing-the-three-dimensional-structure-of-a-molecule.html>

<https://in.mathworks.com/help/bioinfo/sequence-alignment.html>



Protein Aggregation

(Currently Active Area of Research-Good for PhD)

Protein Aggregation

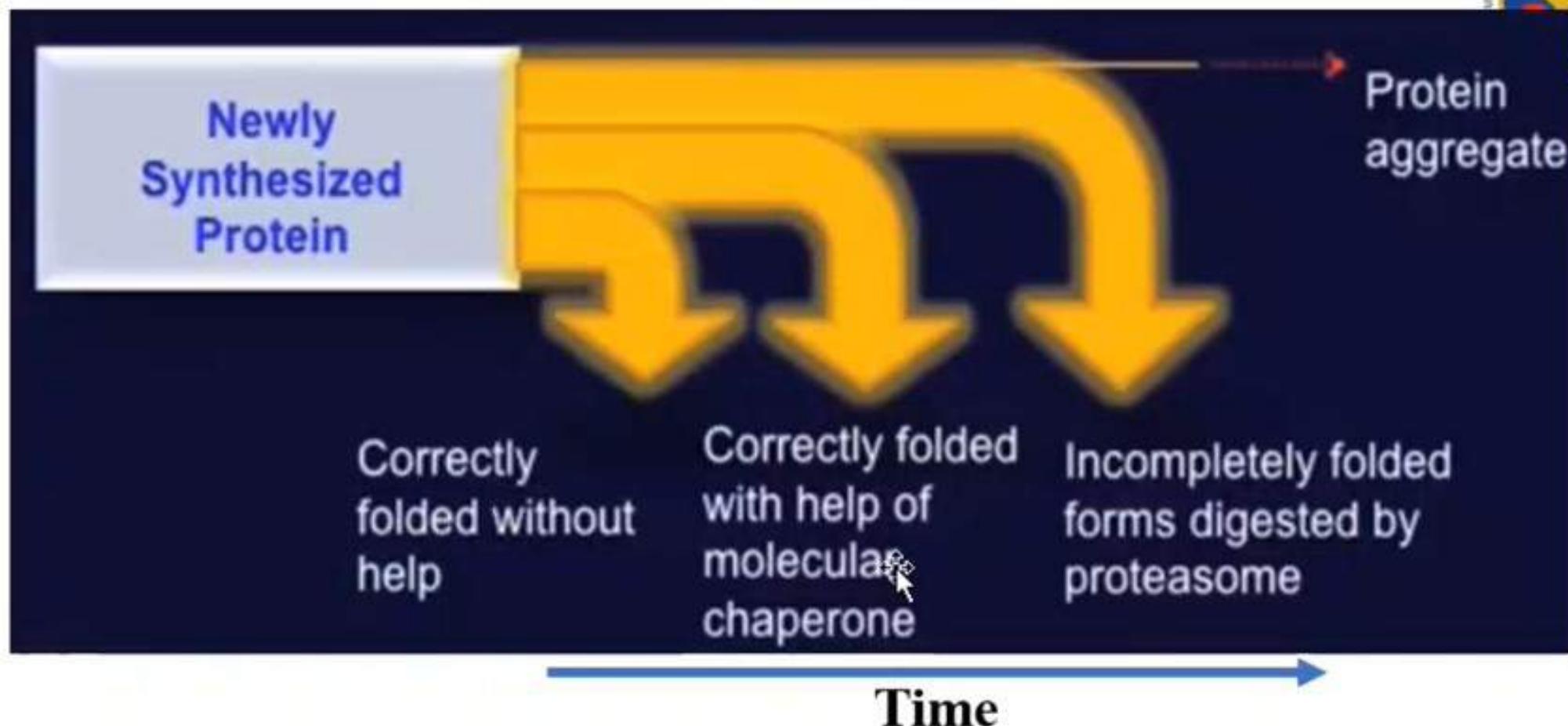
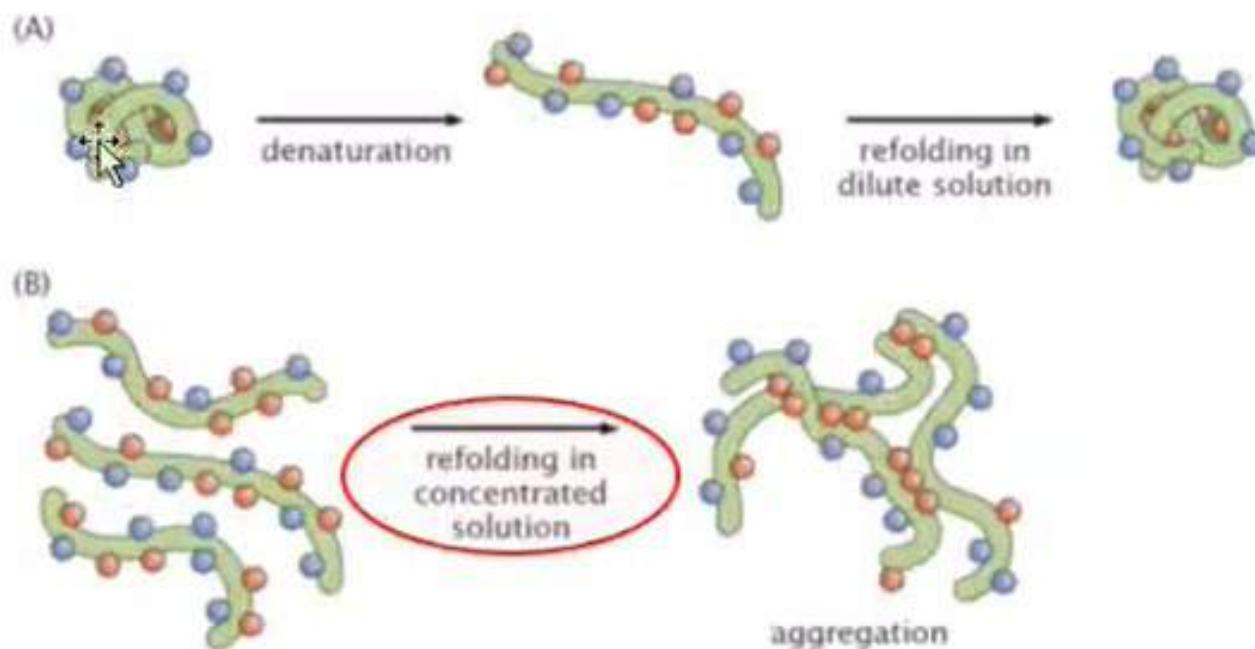


Figure from NPTEL Lecture (Dr.Sanjeeva Srivastava, IIT Bombay)



Protein Aggregation



Several amino acids molecules folding simultaneously nearby results in Aggregation

Figure 14.22: Protein folding and aggregation. A protein folded in its native state sequesters hydrophobic domains on the inside to hide the hydrophobic core. Denaturation disrupts the native structure, exposing these hydrophobic patches. (A) When the protein is allowed to refold in very dilute solution, the hydrophobic patches within a single molecule self-associate to reform the native hydrophobic core. (B) At high concentration, the hydrophobic patch of one protein molecule may associate with the hydrophobic patch of another, triggering protein aggregation rather than native refolding. Hydrophobic residues are shown in red, while hydrophilic residues are shown in blue.

Figure/Content from Rob Phillips "Physical Cell Biology"

Protein Aggregation

- Aggregation occurs when many amino chains expose their hydrophobic residues and interact.
- Aggregation is very hard to reverse
- Aggregation mechanism depend on protein sequence/environment
(Different for different people/disorders)
- Aggregates are risk for immune response.

Active Research Groups in Protein Aggregation

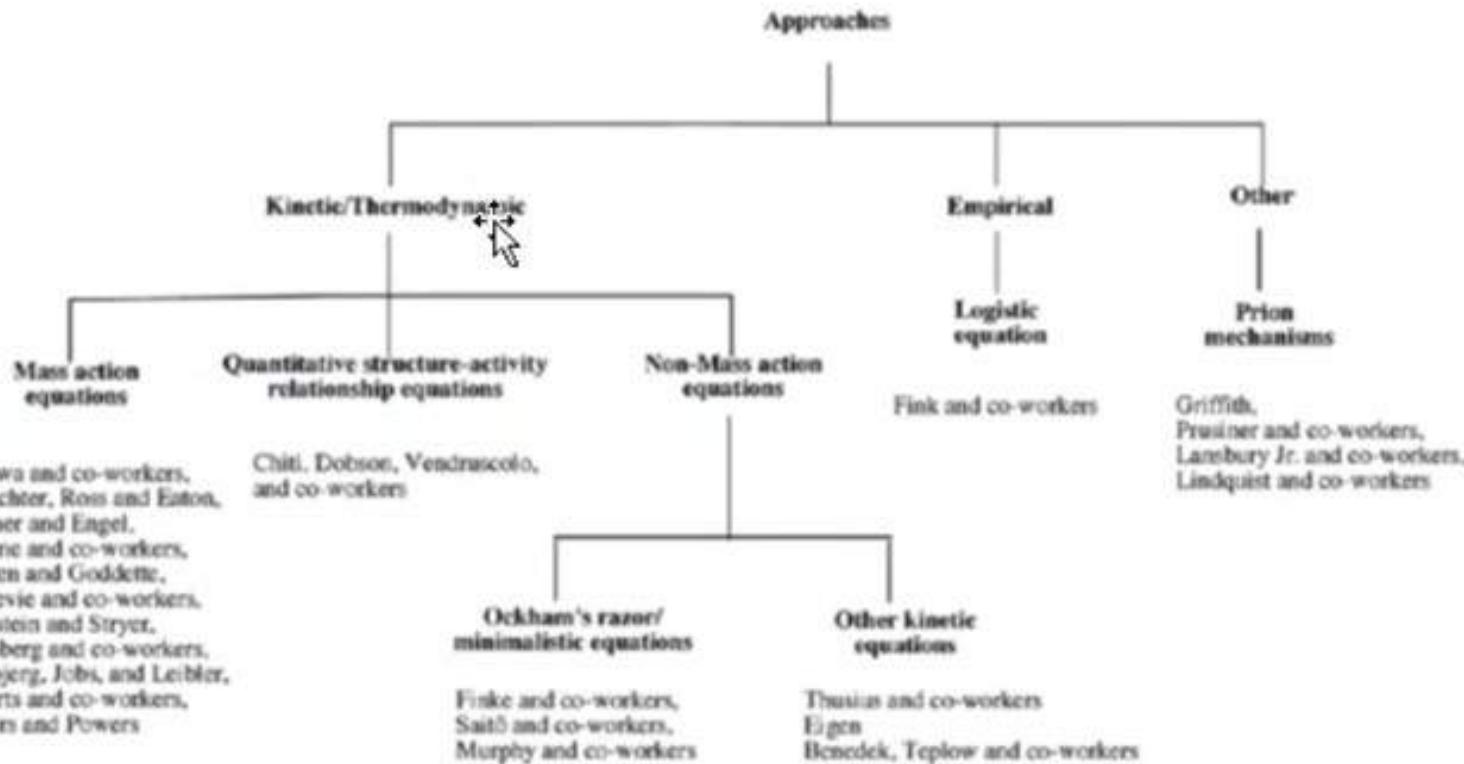
Review

Protein aggregation kinetics, mechanism, and curve-fitting: A review of the literature

Aimee M. Morris, Murielle A. Watzky *, Richard G. Finke *

Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

A.M. Morris et al. / Biochimica et Biophysica Acta 1794 (2009) 375–397



Scheme 2. Literature approaches to determining the kinetics and mechanism for protein aggregation.

Chaperones to avoid Protein Aggregation

Chaperones and Protein Aggregation

- Chaperons increase the efficiency of protein folding by avoiding unfavorable folding paths.
- Typical functions
 - To prevent aggregations.
 - To prevent interference

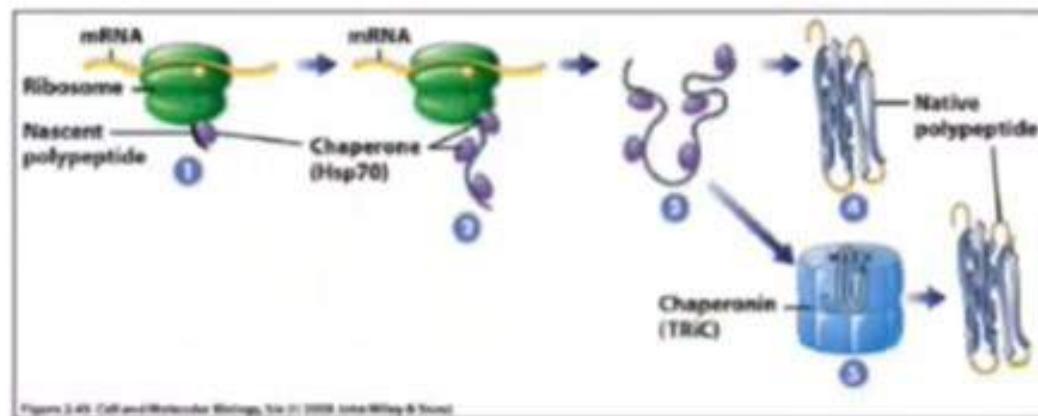
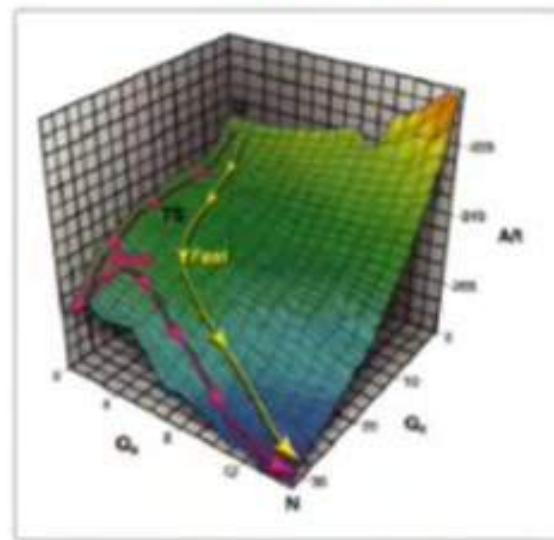
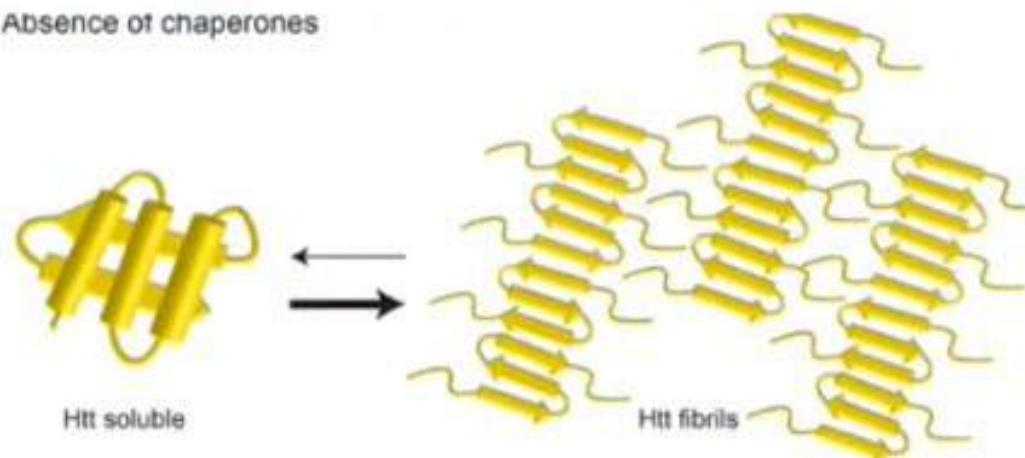


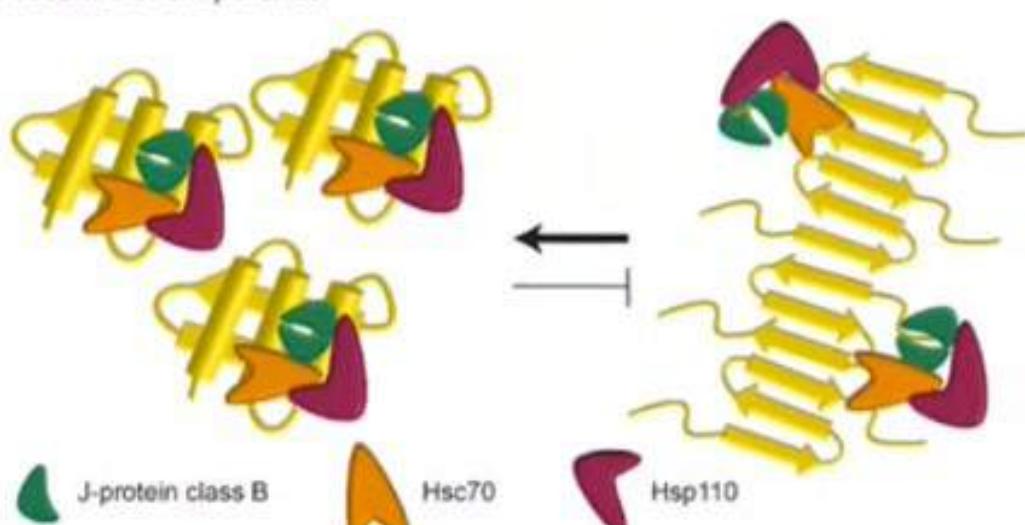
Figure 3.49 Cell and Molecular Biology, 8e (c) 2008 John Wiley & Sons

Chaperones for Protein Folding (Huntington disease)

Absence of chaperones



Presence of chaperones



Complete suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex

Annika Scior, Alexander Buntru, Kristin Arnsburg, Anne Ast, Manuel Iburg, Katrin Juenemann, Maria Lucia Pigazzini, Barbara Mlody, Dmytro Puchkov, Josef Priller, Erich E Wanker, Alessandro Prigione, Janine Kirstein

Author Affiliations

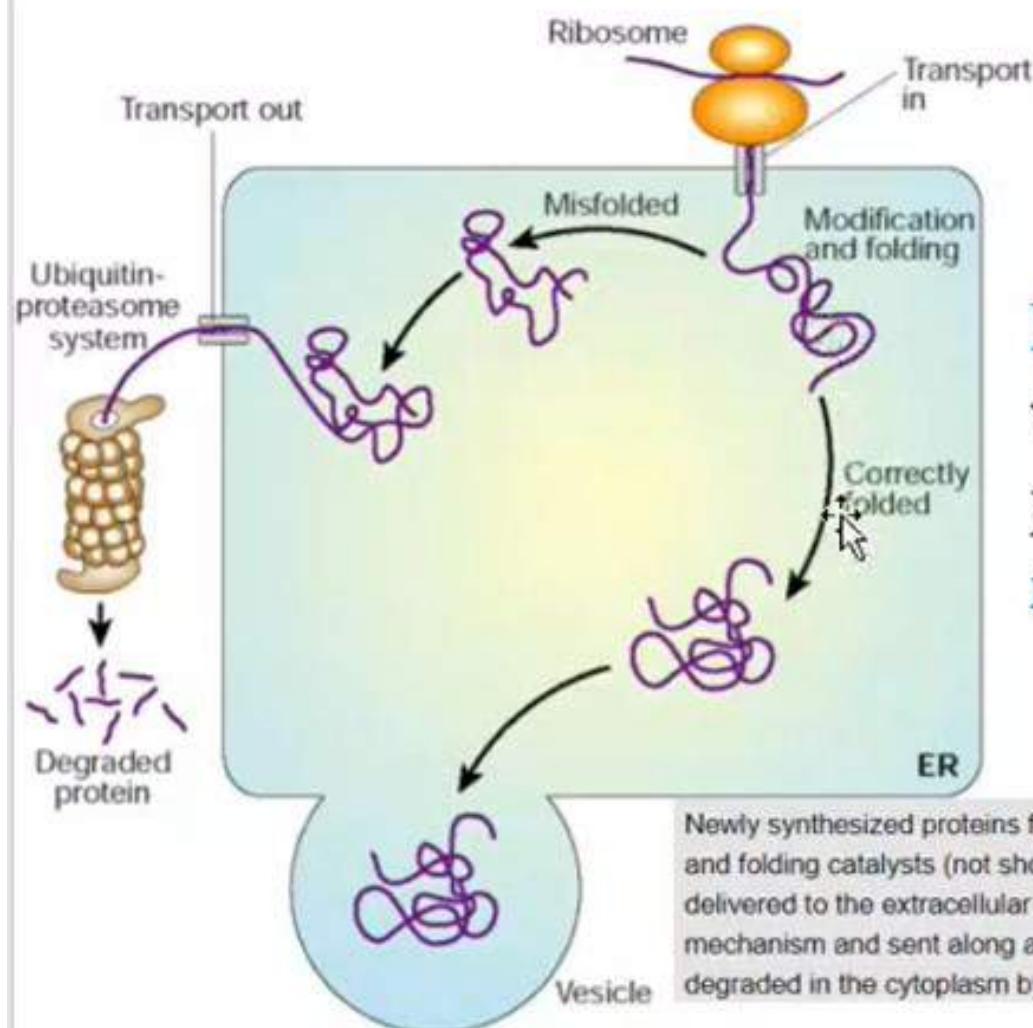
DOI 10.1525/embj.201797212 | Published online 06.12.2017
The EMBO Journal (2018) 37, 282-299

Chaperones suppressed the build-up of pathogenic amyloid (Htt) fibrils and disaggregates them

Huntington's is an inherited disease that causes the progressive breakdown (degeneration) of nerve cells in brain



Protein Ingestion by proteasome



Proteasomes are protein complexes which degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds

Newly synthesized proteins fold into their three-dimensional structures with the help of a series of molecular chaperones and folding catalysts (not shown). Correctly folded proteins are then transported to the Golgi complex and from there delivered to the extracellular environment. However, incorrectly folded proteins are detected by a quality-control mechanism and sent along another pathway (the unfolded protein response), in which they are ubiquitinated and then degraded in the cytoplasm by proteasomes.

Aggregate Versus Folded Protein (Energy Funnel)

Review Article | Published: 20 July 2011

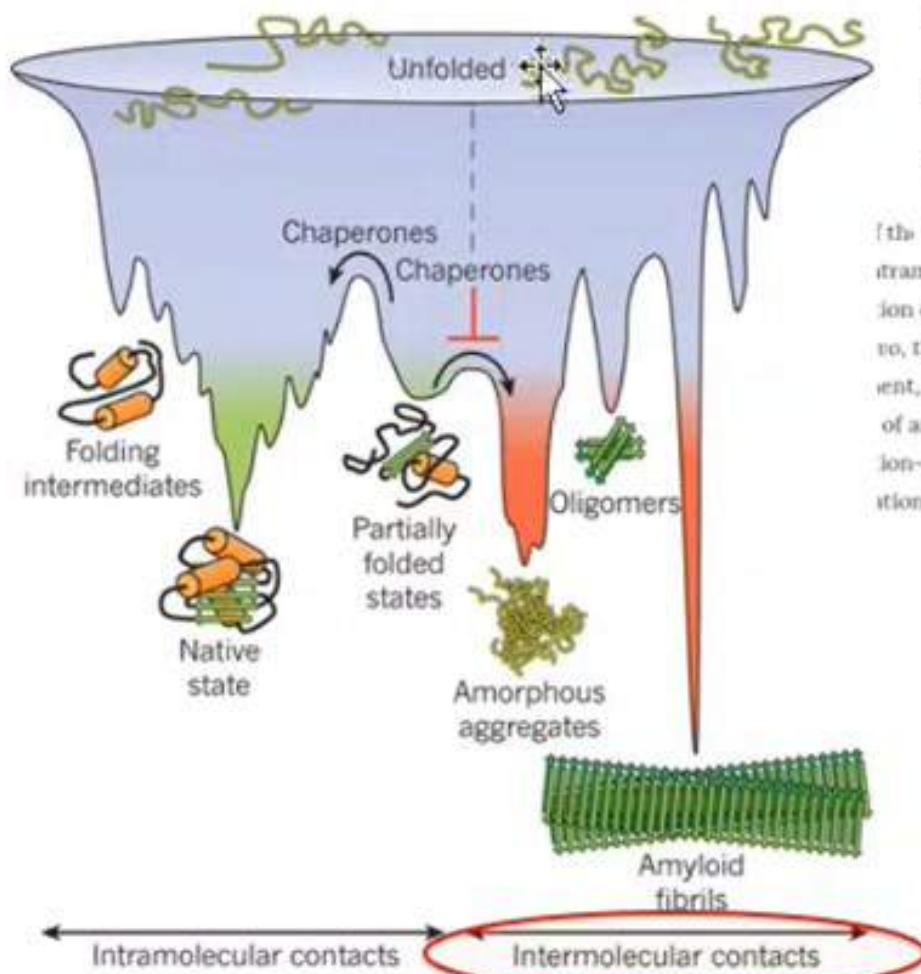


Molecular chaperones in protein folding and proteostasis

F. Ulrich Hartl, Andreas Bracher & Manajit Hayer-Hartl

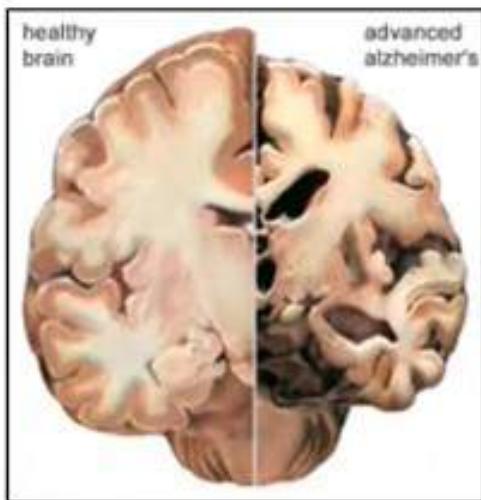
10.1038/nature10212 | Download Citation

In the free-energy landscape of protein folding, the native state (green) is at the bottom of a deep well, while unfolded states (yellow) are at the top of the funnel. Chaperones (red) bind to partially folded states (orange) and facilitate their transition to the native state. Other pathways include the formation of folding intermediates (orange), amorphous aggregates (green), oligomers (green), and amyloid fibrils (green). Intramolecular contacts (blue arrows) drive the folding process, while intermolecular contacts (red oval) can lead to aggregation.



Several amino acids molecules folding simultaneously nearby

Alzheimer's (Introduction)



Symptoms
similar to
ageing

Chronic neurodegenerative disease



Protein Aggregation,
Age, multilingualism,
Genetics, Brain Gray Matter etc.



Multiple
Factors



Loss in memory, loss in attention,
Confusion about places, Trouble
completing tasks etc.



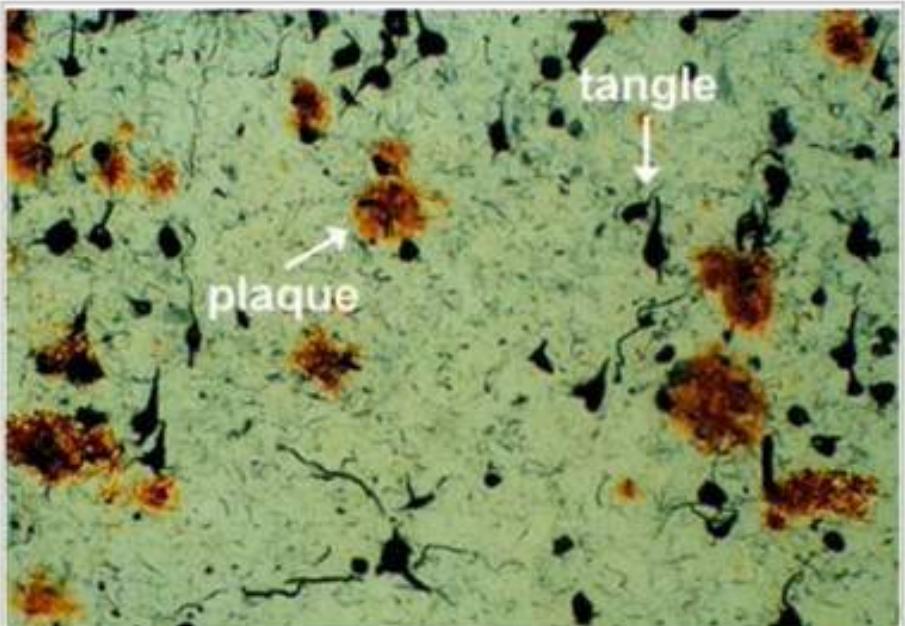
No Medication



Life
expectancy
2-9 years

Content Integrated from various sources/articles

Alzheimer's (Proteins Involved)



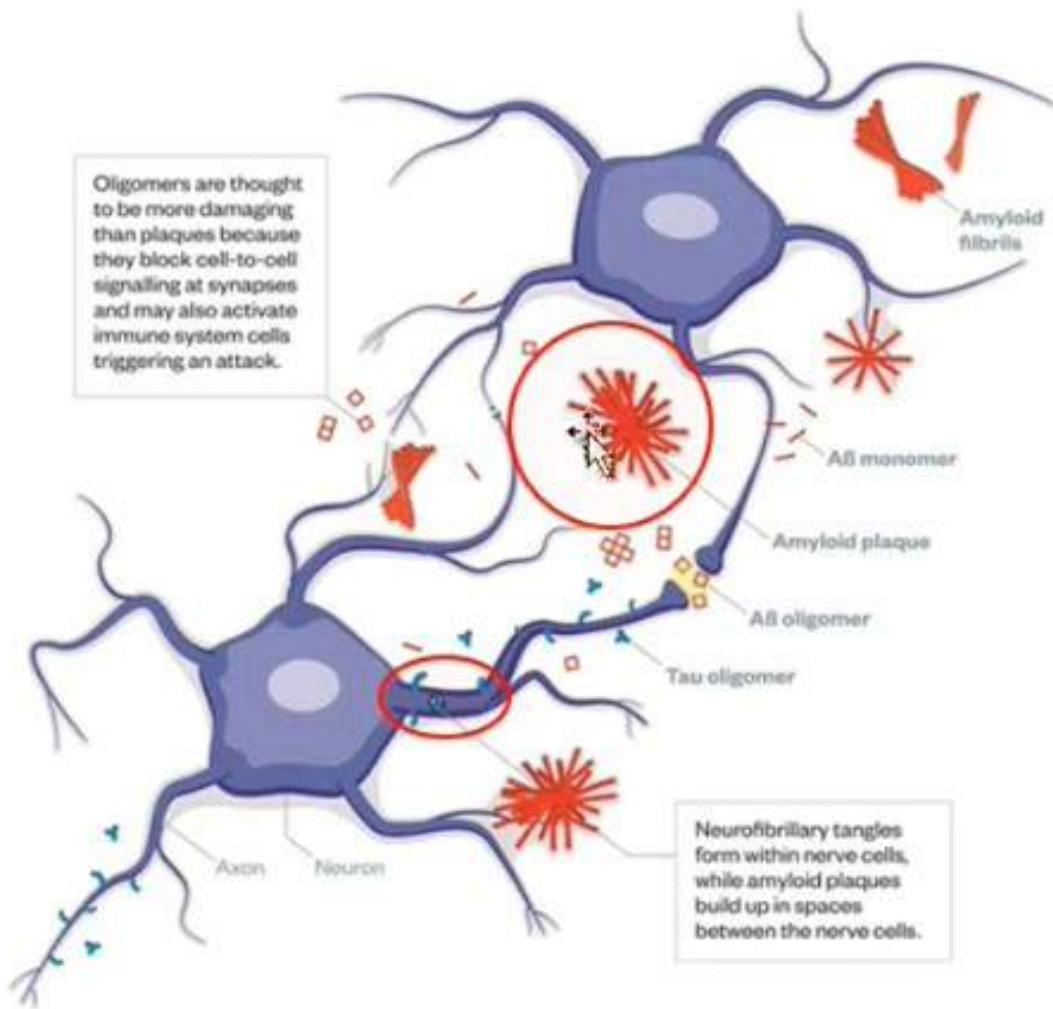
Histopathology of brain
showing amyloid plaques
and neurofibrillary tangles (Post mortem)

- Amyloid plaques
(Alpha beta proteins)
- Neurofibrillary tangles
(Tau proteins)

The pharmaceutical journal

<http://www.pharmaceutical-journal.com/attachment?storycode=20069081&atttype=P&atcode=1069155>

Effect of Protein Aggregates (circled) on neural synapse in Alzheimer's



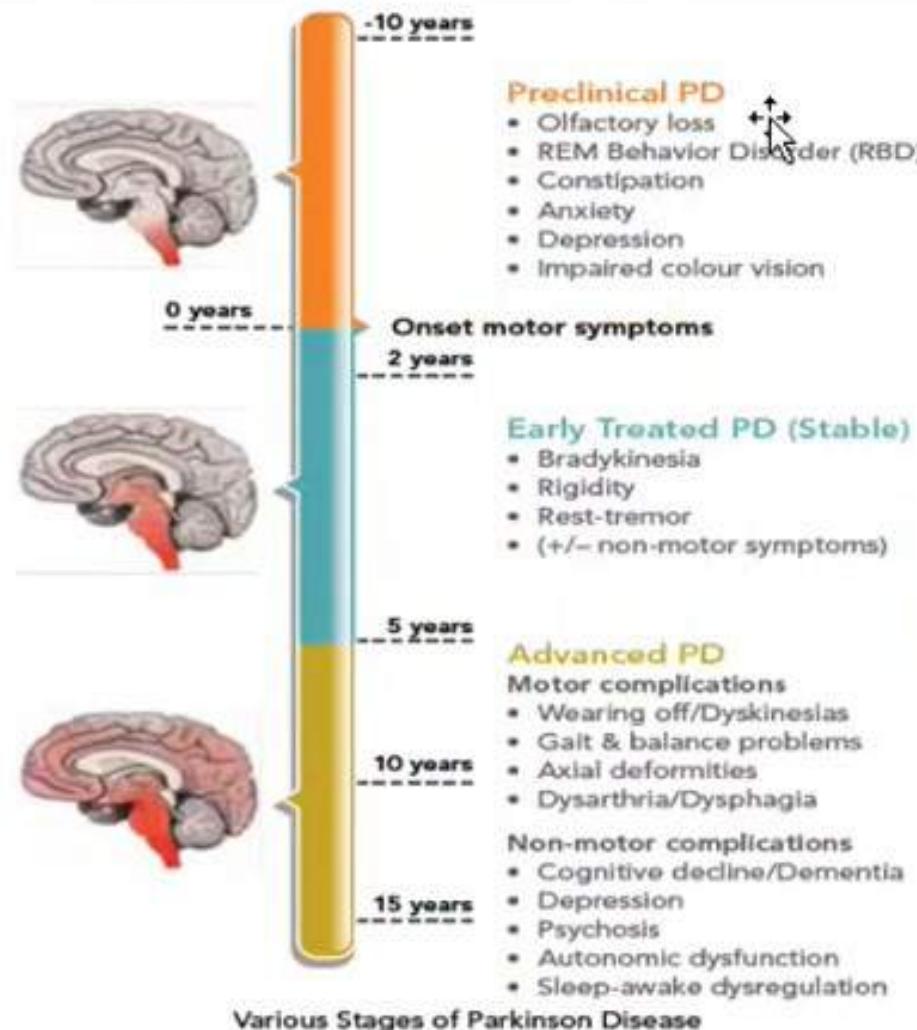
The pharmaceutical journal

<http://www.pharmaceutical-journal.com/attachment?storycode=20069081&atttype=P&atcode=1069155>

ALZHEIMERS CURRENT TREATMENT (Mayoclinic, USA)

- Alzheimer's disease can't be cured as of today, but medications can help control your symptoms (unfortunately with side effects)
- **Memantine (Namenda)**: This drug works in another brain cell communication network and slows the progression of symptoms with moderate to severe Alzheimer's disease.
- Inhibitors include **donepezil (Aricept)**, **galantamine (Razadyne)**

Parkinson's (Stages)



<https://www.singhealth.com.sg/DoctorsAndHealthcareProfessionals/Medical-News/2014/Pages/Parkinson-Disease-Likely-Treatment-Options.aspx>

Parkinson's (In Pictures)

Tremor



Tremor of one hand is an early manifestation of parkinsonism



Tremor often improves or disappears with purposeful function

Bradykinesia



Difficulty in performing simple manual functions may be initial symptom



Late Stage Disabilities



Stage 4: significant disability; limited ambulation with assistance



Stage 1: unilateral involvement; blank facies; affected arm in semiflexed position with tremor; patient leans to unaffected side



Stage 2: bilateral involvement with early postural changes; slow shuffling gait with decreased excursion of legs



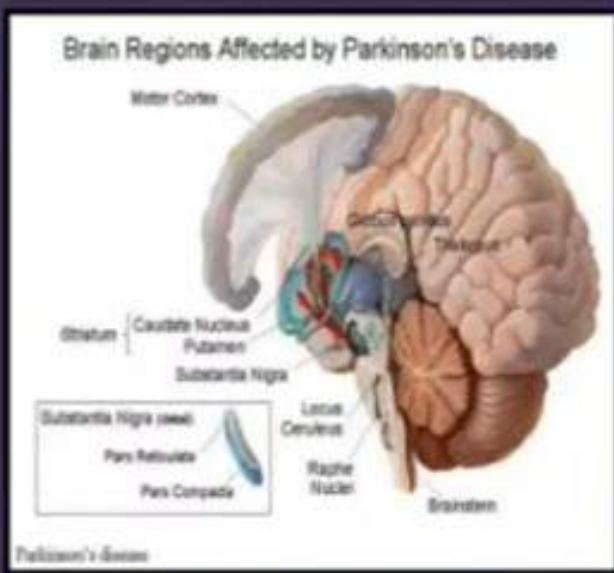
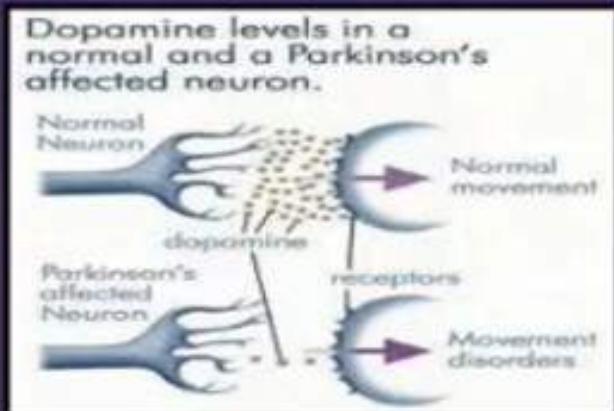
Stage 3: pronounced gait disturbances and moderate generalized disability; postural instability with tendency to fall

F. Noller
JOHN A. CRAIG, MD
C. Machado



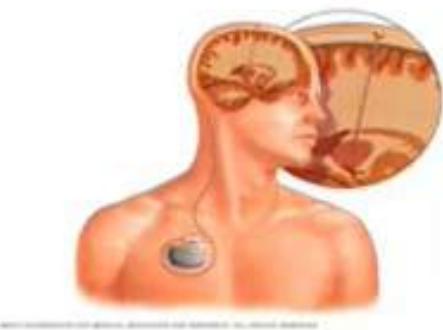
Stage 5: complete invalidism; patient confined to bed or chair; cannot stand or walk even with assistance

What happens in Parkinson's Disease?



PARKINSONS CURRENT TREATMENT (Mayoclinic, USA)

- Parkinson's disease can't be cured, but medications can help control your symptoms, often dramatically
- Levodopa, the most effective Parkinson's disease medication, is a natural chemical that passes into your brain and is converted to dopamine
- Deep brain stimulation (DBS), surgeons implant electrodes to stimulate specific part of your brain.
- Green Tea/Caffeine reduce parkinson occurrence



NEURODEGENERATIVE DISEASE AND CHARACTERISTICS

Table 1. Late-onset neurodegenerative diseases and some of their characteristics.

Disease	Etiology; Incidence	Main neuron population affected	Characteristic pathology	Symptoms
Alzheimer's disease	Sporadic 95% Familial ≤5% (including APP, PSEN1/2); 1275/100 000 (>65 years)	Acetylcholinergic neurons; degeneration in the entorhinal cortex, spreading to the temporal lobe and frontal cortex	Extracellular β- amyloid plaques, intracellular neurofibrillary tangles	Cognitive dysfunction with memory loss.
Parkinson's disease	Sporadic 90-95% Familial 5-10% (including α-syn, parkin, PINK, DJ-1, LRKK2); 160/100 000 (>65 years)	Dopamine neurons in the substantia nigra pars compacta	Lewy bodies containing aggregated α-syn	Resting tremor, postural instability, rigidity, akinesia/ bradykinesia
Huntington's disease	Monogenic (huntingtin); 0.3-0.8/100 000 (all ages)	GABAergic medium spiny neurons in the striatum	Aggregated huntingtin	Chorea, psychiatric disturbances, cognitive impairment
Amyotrophic lateral sclerosis	Sporadic 90-95% Familial 5-10% (including SOD1, C9orf72; TDP-43, FUS, UBQLN2); 1.6/100 000 (all ages)	Lower motor neurons in the spinal cord, upper motor neurons in the cortex	Cytoplasmic ubiquitinated aggregates of SOD1, FUS or TDP-43	Progressive muscle weakness, muscular atrophy, spasticity

GENE THERAPY FOR NEURODEGENERATIVE DISEASE

Since the neurodegenerative diseases are chronic progressive diseases, long-term delivery of therapeutic agents is probably a requisite. This can be achieved, e.g., by continuous protein infusions or by gene therapy. In animal models of neurodegenerative diseases, several approaches using gene therapy have proven to be beneficial. In these studies, researchers have tried to alter neuronal networks by modulating the synthesis of neurotransmitters (Björklund and Kordower, 2010), or aimed at neuroprotection by removing pathogenic proteins (San Sebastian et al., 2013), or by supplying the diseased tissue with neurotrophic support (Allen et al., 2013).

ELIMINATION OF PATHOGENIC PROTEIN

2.4.2 ELIMINATION OF PATHOGENIC PROTEIN

The idea that misfolded abnormal proteins would be a trigger of pathogenesis in neurodegenerative diseases would suggest that by removing these pathogenic proteins, neuroprotection could be achieved (for overview of aggregates typical to each disease, see Figure 9). The elimination or reduction of abnormal proteins can be done either by silencing the expression or by increasing the clearance of the proteins (San Sebastian et al., 2013).

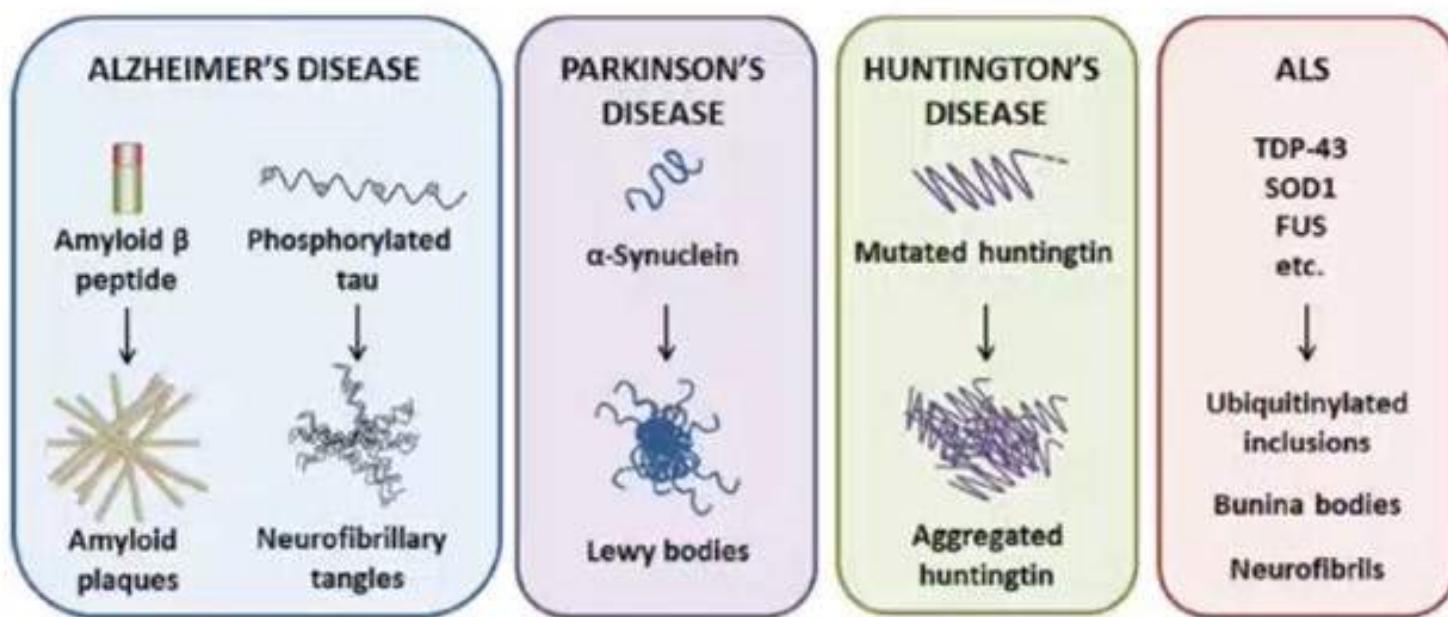
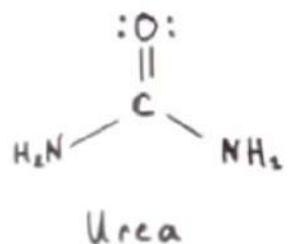


Figure 9 Protein aggregation in late-onset neurodegenerative diseases.

ANFINSENS EXPERIMENT

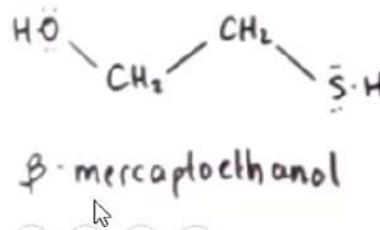
Anfinsen's Experiment

In 1950's, Christian Anfinsen conducted a series of experiments that ultimately showed that the information needed to form the three-dimensional active protein lies in its sequence of amino acids. Later experiments generalized this idea that the primary structure determines the conformation of the protein.



- Urea readily disrupts non-covalent bonds such as ionic interactions and hydrogen bonds.

Denaturing agents which break protein molecule



- β -mercaptoethanol breaks down disulfide bonds via an oxidation-reduction reaction.

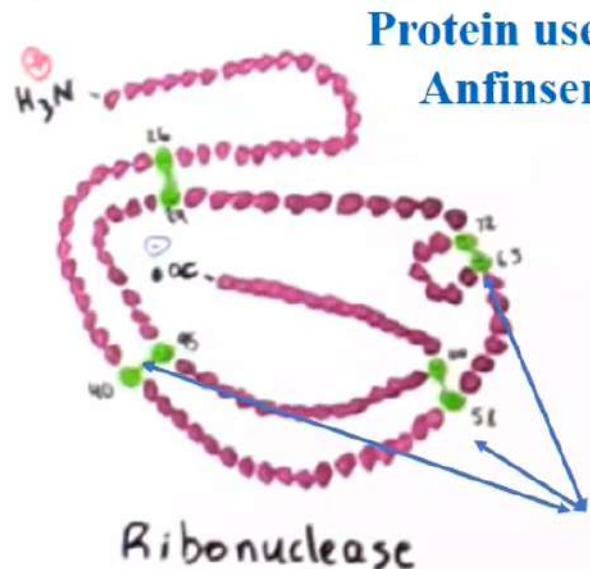




ANFINSENS EXPERIMENT

Overview:

- Christian studied a particular type of enzyme called ribonuclease. This protein consisted of 124 amino acids and had a tertiary structure with four disulfide bonds.



Protein used by
Anfinsen's

- The plan was to destroy the tertiary structure of ribonuclease by using appropriate agents and then investigate the conditions under which the proper tertiary structure reformed.

Disulphide bonds



ANFINSENS EXPERIMENT

Experiment # 1:



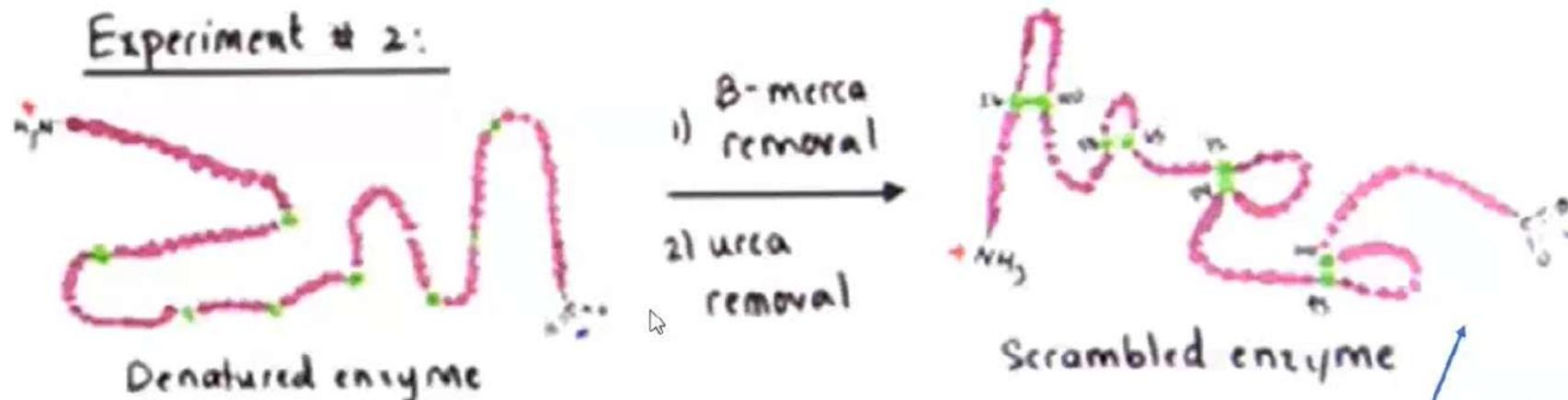
- When the native enzyme was treated with excess β -mercaptoethanol and 8.0 M urea, all disulfide bonds and hydrogen bonds were broken and the protein denatured. When the denaturing agents were removed, the enzyme eventually reformed its original tertiary structure.

Agents removed: Disulphide bonds reformed by interaction of denatured protein with oxygen

ANFINSENS EXPERIMENT



Experiment # 2:



- When β -mercaptoethanol was removed first, an inactive enzyme was formed. This is because the improper disulfide bonds were formed.

Primary sequence of amino acids in a polypeptide chain dictate the type of noncovalent interactions for formation of disulphide bonds



ANFINSENS EXPERIMENT



CHETAN BHARTI
MOHAMMAD ATI...

COTA NAVIN GUPTA
VAIBHAV VALLAB...

SIDDARTH GAUT...
KABILAN M...

SHATAKSHI KAUS...
ANINDYA RAJAN

TANMAY TUSHAR...
SURYASHI SHREE

RAJESH MUHAL
MAITREYEE NITI...

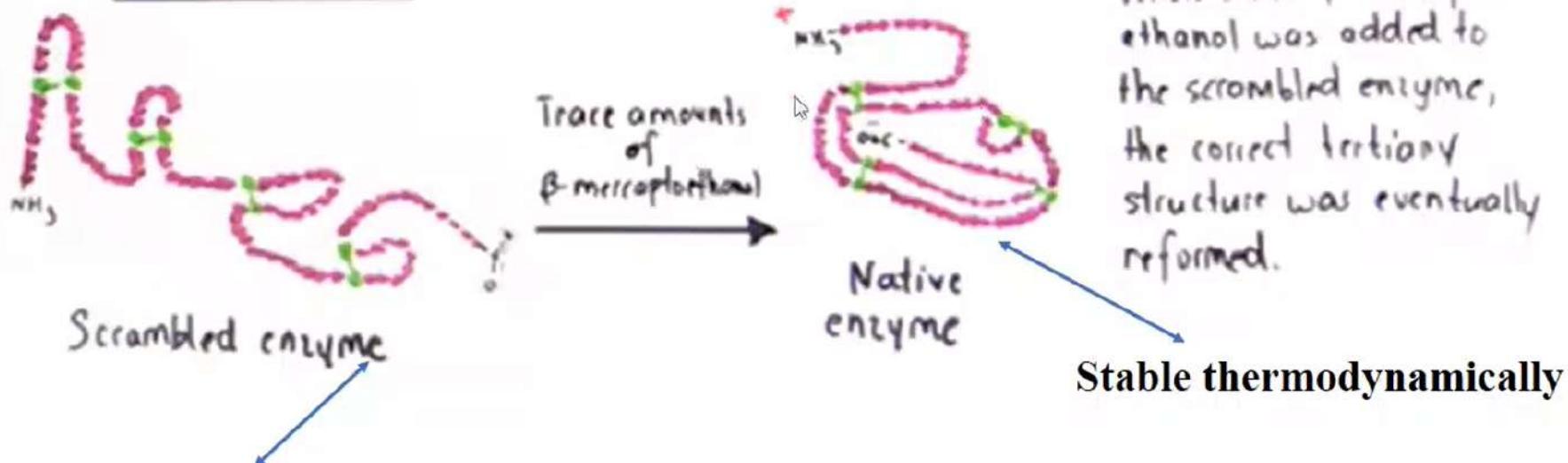
AMEY RAMESH R...
VISESH BARATAM

ADITYA BAJAJ
BOBBY KUMAR

KUMAR SHASHW...

+14

Experiment # 3:



Scrambled Enzyme is placed in a test tube with β -mercaptoethanal

Levinthal's paradox

ROBERT ZWANZIG, ATTILA SZABO, AND BIMAN BAGCHI*

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Building 2, National Institutes of Health, Bethesda, MD 20892

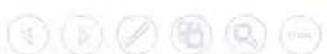
Contributed by Robert Zwanzig, October 7, 1991

How long does it take for a protein to fold up into its native structure? In a standard illustration of the Levinthal paradox, each bond connecting amino acids can have several (e.g., three) possible states, so that a protein of, say, 101 amino acids could exist in $3^{100} = 5 \times 10^{47}$ configurations. Even if the protein is able to sample new configurations at the rate of 10^{13} per second, or 3×10^{20} per year, it will take 10^{27} years to try them all. Levinthal concluded that random searches are not an effective way of finding the correct state of a folded protein. Nevertheless, proteins do fold, and in a time scale of seconds or less. This is the paradox.



Protein Unfolding (EGG)

- Increase Temperature (except primary structure all higher forms broken)
- Add Vinegar (disruption of ionic bonds- tertiary, quartenary broken)
- Chemicals (add alcohol) (except primary structure all higher forms broken)
- Enzymes (lets say we eat the egg then digestive system breaks it into amino acids for our body to absorb it).



Article

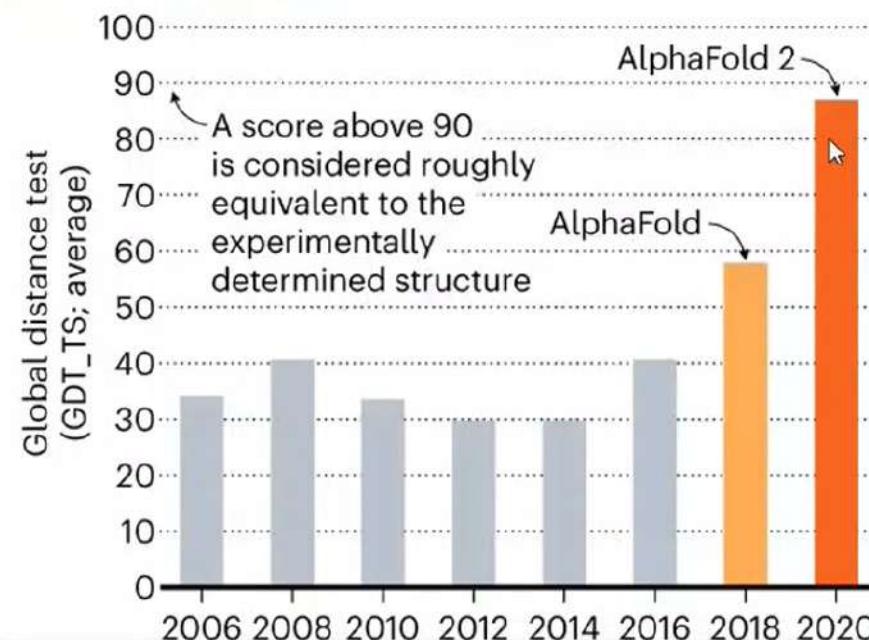
Highly accurate protein structure prediction with AlphaFold

In some cases, AlphaFold's structure predictions were indistinguishable from those determined using 'gold standard' experimental methods such as X-ray crystallography and, in recent years, [cryo-electron microscopy \(cryo-EM\)](#). AlphaFold might not obviate the need for these laborious and expensive methods – yet – say scientists, but the AI will make it possible to study living things in new ways.

STRUCTURE SOLVER

DeepMind's AlphaFold 2 algorithm significantly outperformed other teams at the CASP14 protein-folding contest — and its previous version's performance at the last CASP.

<https://github.com/deepmind/alphafold/>



Cyto



Greek = Kytos
“hollow basket”
meaning CELL

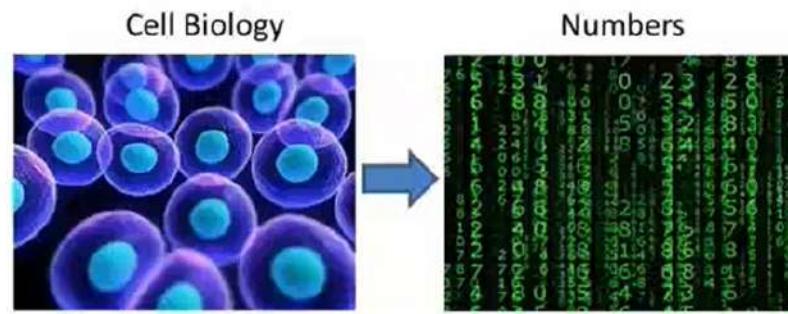
metry



Greek = Metria
“process of
measuring”

WHAT IS CYTOMETRY

- The measurement of **phenotype** at a **single cell** level
- Conducted on a **population** of cells →
- To understand **heterogeneity** in **all systems**



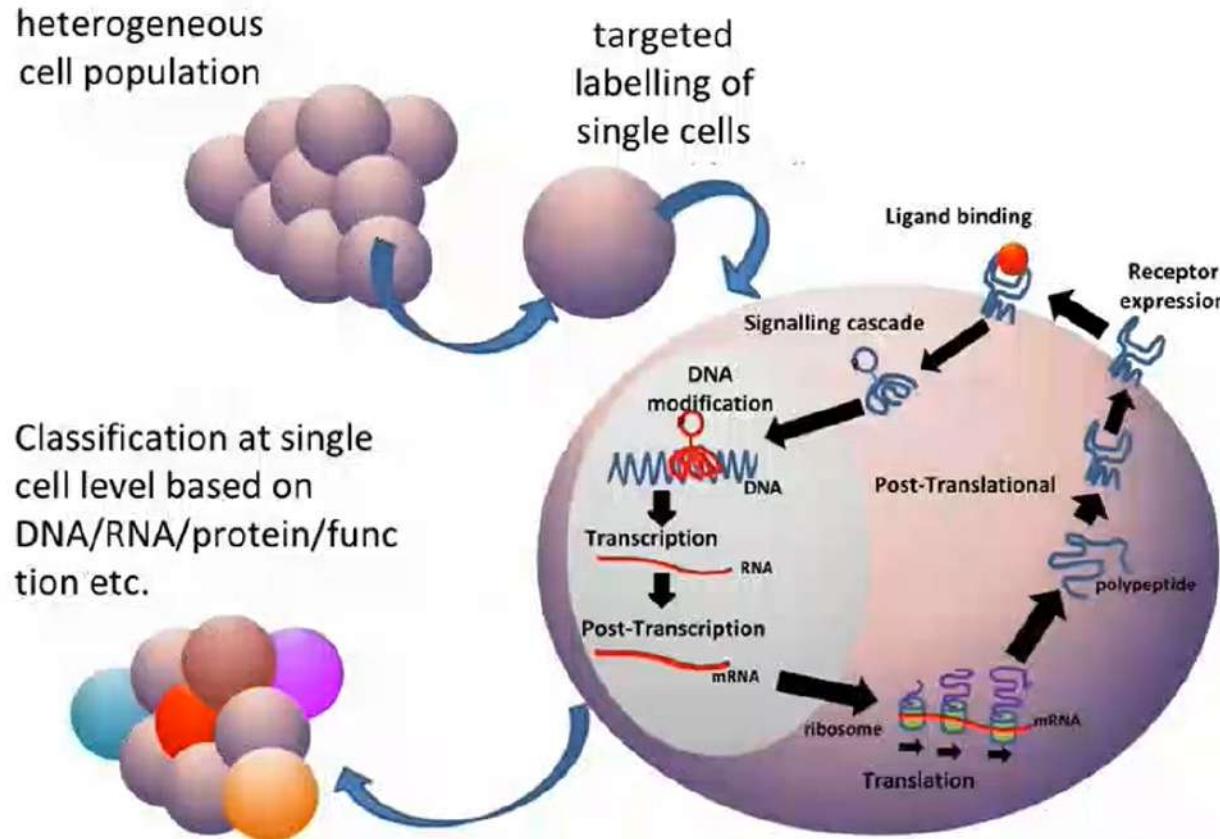
Questions we want to answer with these numbers:

- What types of cells are there and how many?
- What do they do?
- What role do they have in development/disease?



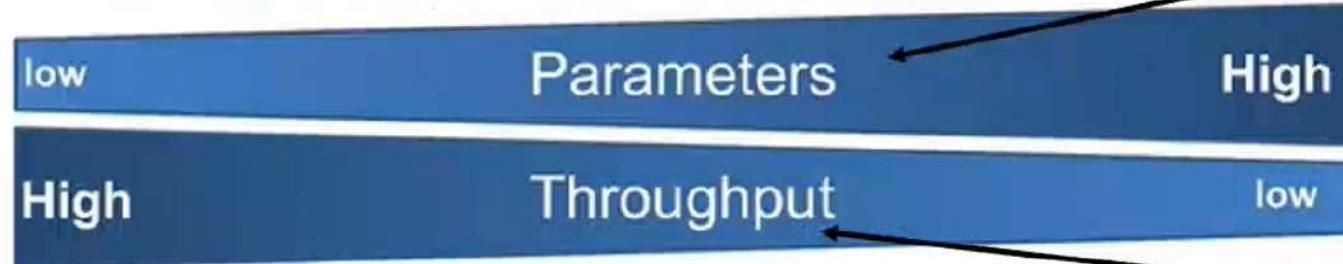
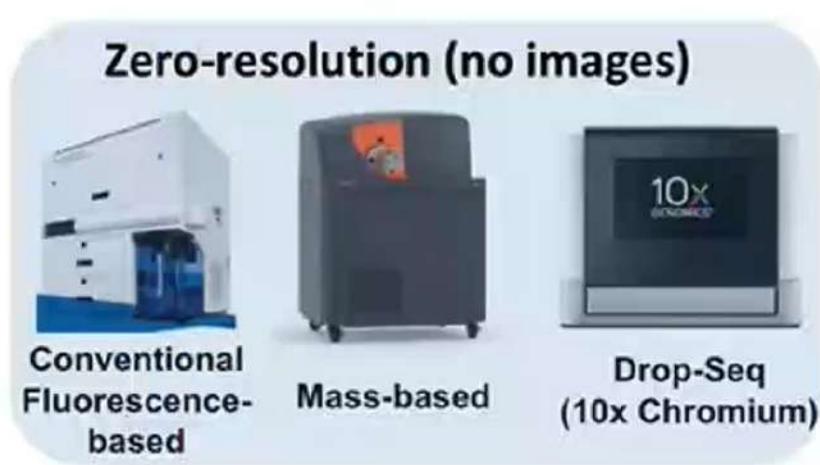
KUMAR SHASHW...

Cytometry is the study of **EVERYTHING** single cell



Cytometers:

The machines we use to measure the cells



Cell related



The underlying principles of Flow Cytometry

Fluidics

- Any Flow Cytometer is built around the unique principle of carrying particles of interest to a point of interrogation by means of a stable stream of fluid.
- Fluids are very difficult to compress and provide excellent stability of motion!



Flickr: Grand Canyon National Park

Apply pressure to Fluid and keep pressure constant over time, then Flow is constant

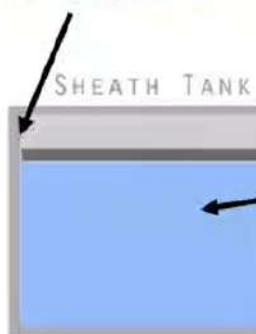
Flow is volume per time (Happens in RIVERS)



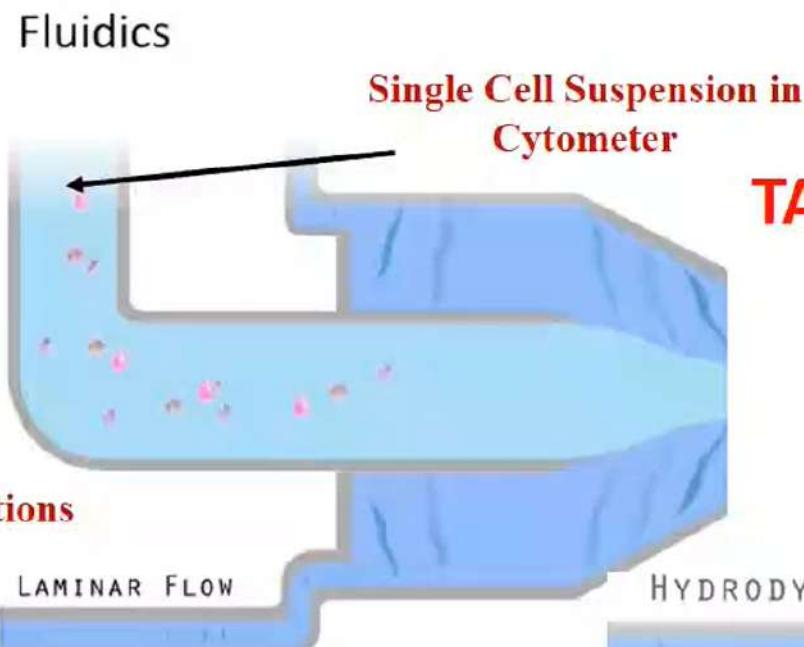
KUMAR SHASHW...

Hydrodynamic Focussing

Constant Pressure over volume



Stage 1

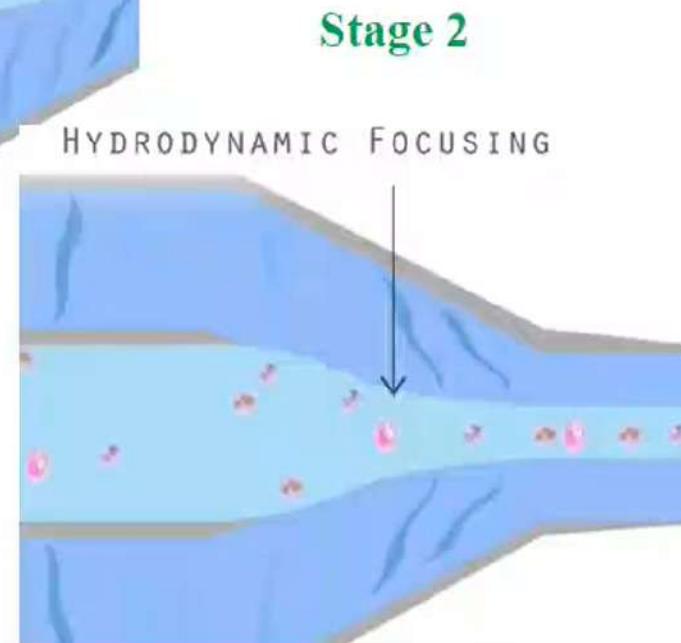


Fluid system which drives our cells forward at discrete pace with constant speed

Any particle injected will align in the middle of the stream

Cells samples focussed in time.

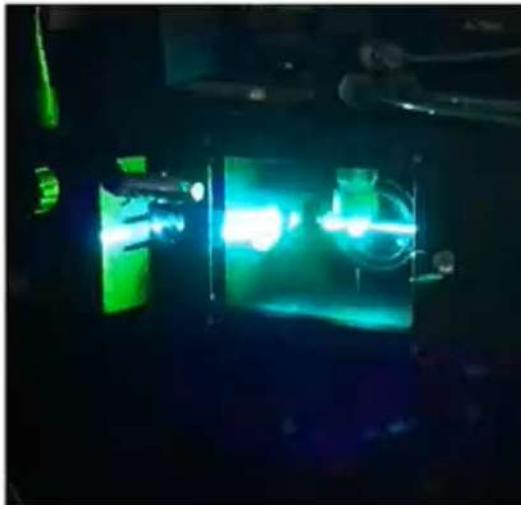
TAKING FLUIDICS TO REALITY



WHAT IS FLOW CYTOMETRY

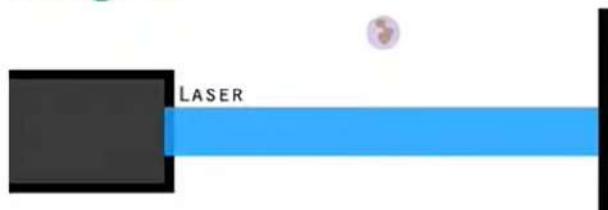
Optics – Signal generation and detection

- Flow Cytometers use lasers as coherent, focused and strong excitation light source to excite fluorescent dyes and provide light for particle scatter measurements
- Flow Cytometers convert fluorescent signals into scalable electric potentials which correlate in strength with the number of emitted fluorescent photons



Optics – Light Scattering

Stage 1

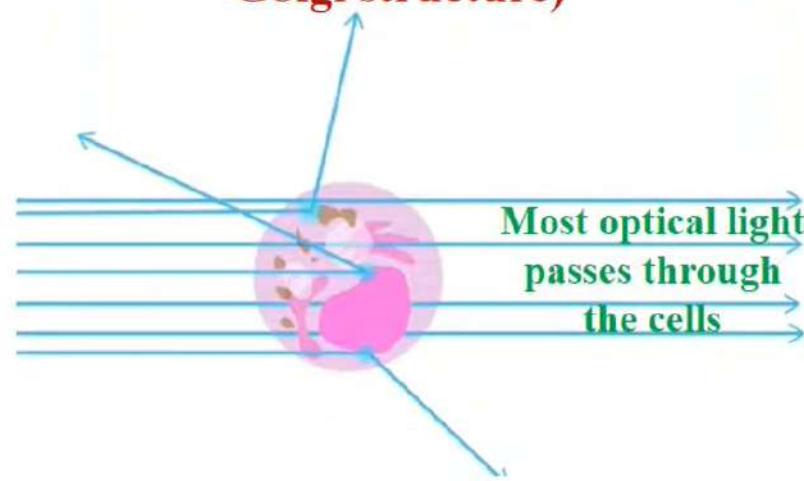


Scattering is omnidirectional

Different optical densities in the cell(structures like nucleus, vesicles, Golgi structure)



Stage 2

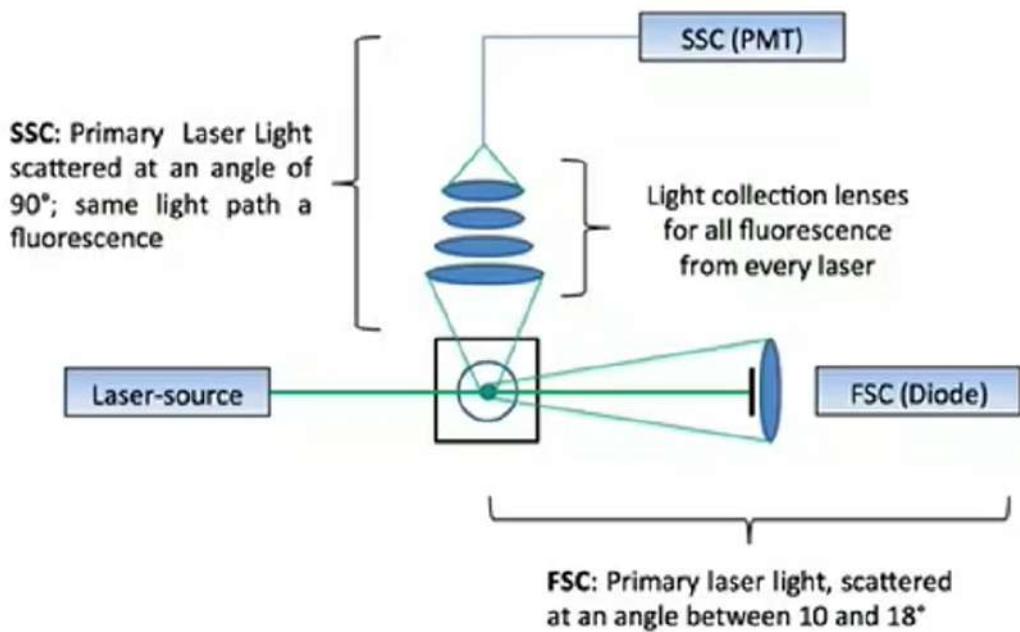


Most optical light passes through the cells

Obscuration bar is a horizontal piece of metal that blocks laser light but allows scattered light to pass over it and into the detector



Optics – Light Scattering



Scattering depends on LASER light, so signals are strong/intense

Light Scattering activity depends on the cells optical density, its roughness and to some extent its size.

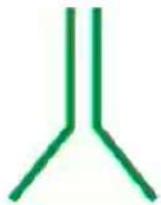


FSC and SSC are both measuring primary scattered laser light

Optics – Detecting Fluorescence

Classic Flow Cytometry uses very specific antibodies to label cellular antigens (extra and intracellular)

The antibodies are labeled in most cases directly with fluorescent dye(s) to generate a fluorescent signal



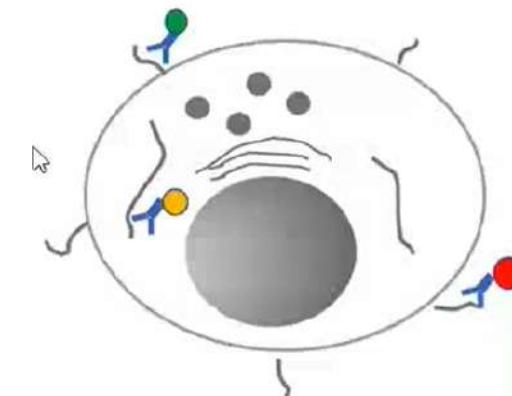
Antibody1-FITC



Antibody2-PE



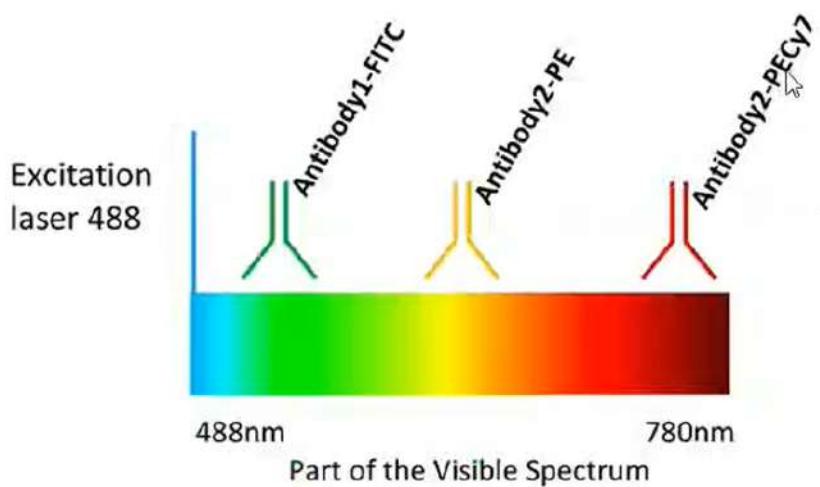
Antibody3-PECy7



Nicely excitable

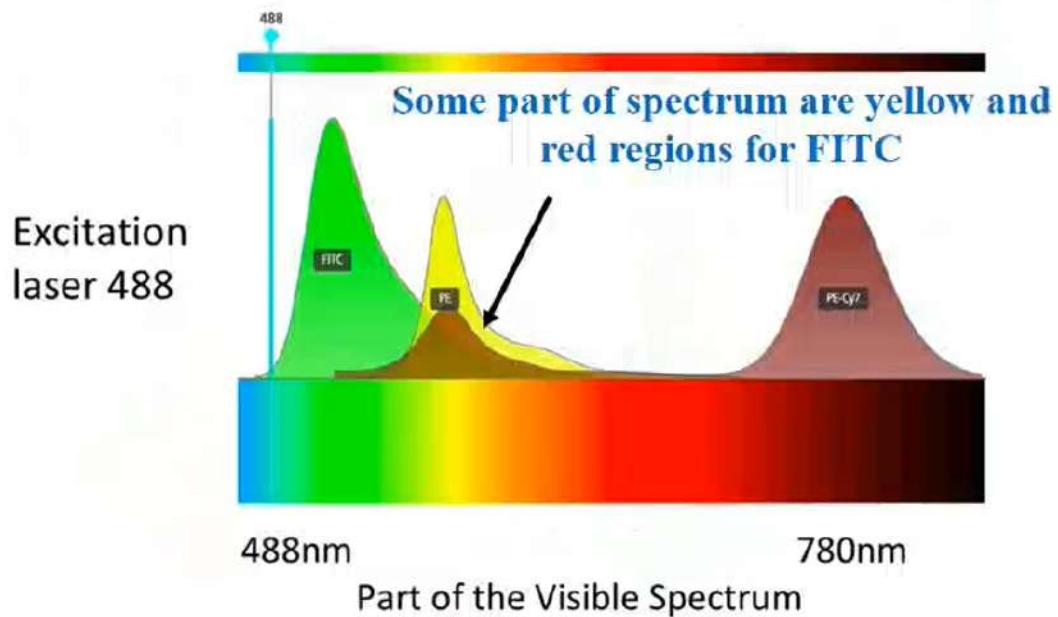


Optics – Detecting Fluorescence



Dyes perfectly spread out

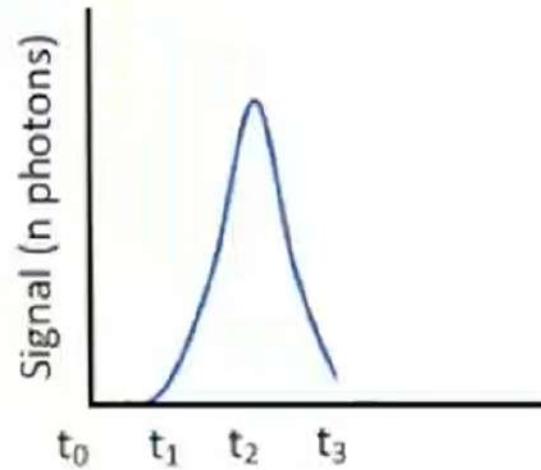
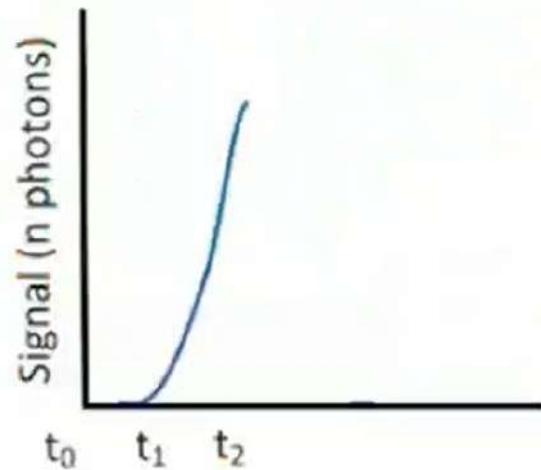
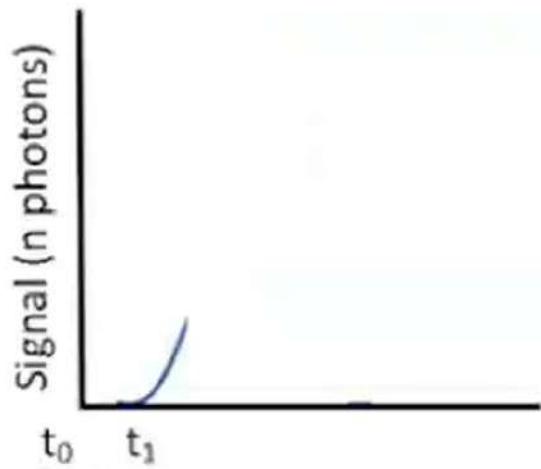
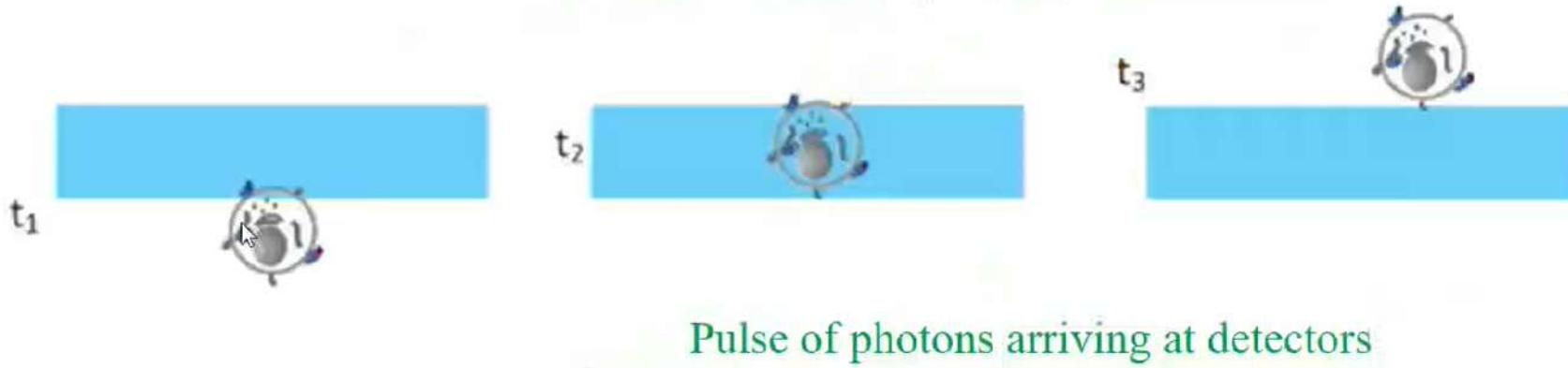
Optics – Detecting Fluorescence



Graphs:https://www.bdbiosciences.com/br/research/multicolor/spectrum_viewer/index.jsp



SIGNAL CONVERSION



Flow Cytometric Analysis of Protein Aggregates

Author(s): Sushanta Debnath, Bikram Nath, Abhijit Chakrabarti*

Journal Name: Protein & Peptide Letters

Volume 24 , Issue 10 , 2017



Abstract:

Background: Misfolding of proteins often leads to aggregation. Accumulation of diverse protein aggregates in various cells, tissue and organs is the hallmark of many diseases, such as Alzheimer's disease and Parkinson's disease.

Objectives: The main objective of this study was to present a novel method of characterization of protein aggregates, associated with differential toxicity with different size and composition *in vitro* using flow cytometry.

Methods: A Beckman Coulter Epics XL flow cytometer with argon ion laser operating at 488 nm was used for flow cytometry analysis. The voltage and the gain settings for individual channels were set at high voltage and gain for the detections of autofluorescence, fluorescence of adsorbed Congo red, forward scattering (FSC) and side scattering (SSC) intensities from the aggregates of proteins and nanoparticles. Each sample was analyzed to characterize and quantify the number of aggregates with a limit of maximum 20,000 events. The flow cytometry data were analyzed using Flowing software version 2.5.1 and Origin 8.0.

Results: Autofluorescence and scattering intensities could distinguish between amyloid and nonamyloid aggregates. Dot plots of both side scattering (SSC) and forward scattering (FSC) intensities also showed characteristic fingerprint of both the types of aggregates when compared with those of well known nanoparticles of oxides of Fe and Cu.

Conclusion: This work reports a novel, simple and robust flow cytometric method of characterization of protein aggregates of different size and composition which would find wider application in characterization of biomolecular aggregates, in general.

