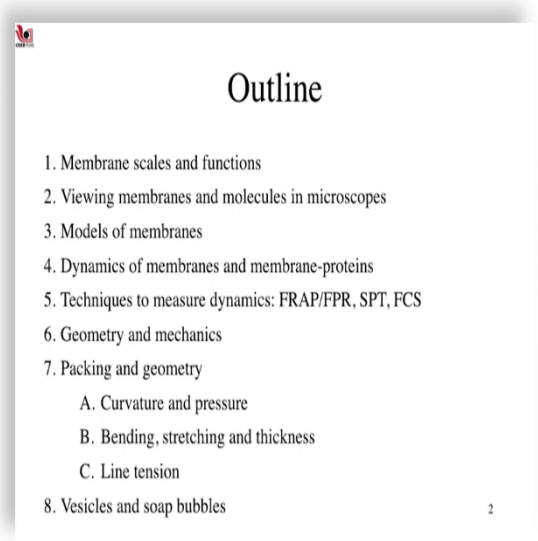
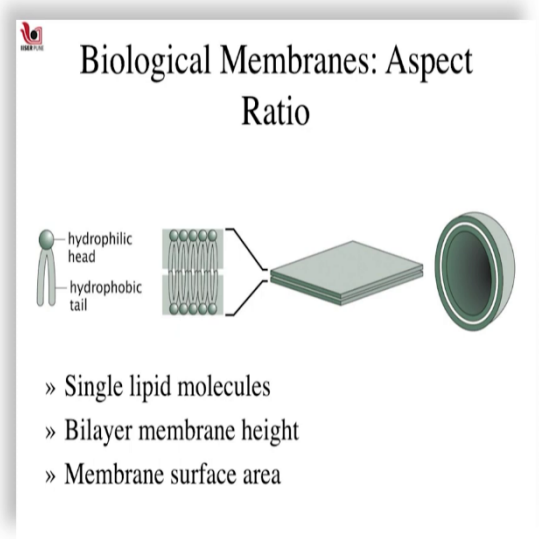
Cellular Biophysics
Doctor Chaitanya A. Athale.
Department of Biology
Indian Institute of Science Eduction and Research, Pune
Lecture 54
Introduction to Membrane Mechanics

(Refer Slide Time: 00:30)



Hi, welcome back. I am going to be talking to you about membrane dynamics and mechanics, in continuation with what I had presented to you, with the idea of perhaps rounding it off and connecting it back to biochemistry and some experimental techniques. So, for today, for this module, I am going to talk about membrane scales functions, how to view them, models, their dynamics of the membranes, and methods to measure the dynamics. And when we return I will talk to you a little bit about geometry mechanics packing, curvature bending stretching line tension vesicles, and so bubbles some of which you have already covered. So let us get to it.

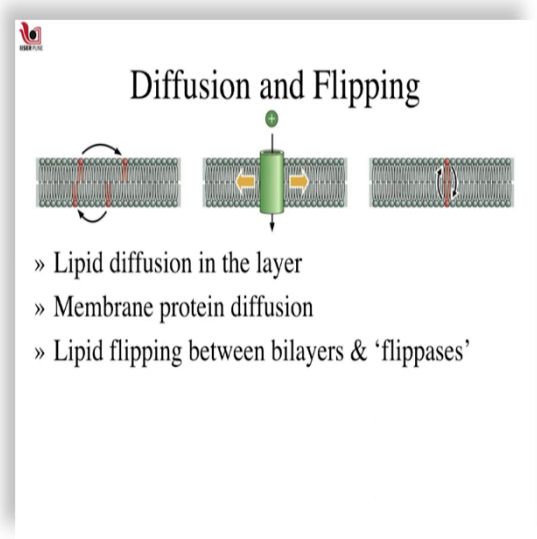
(Refer Slide Time: 00:52)



Biological membranes have a typical aspect ratio, and that makes them slightly unusual. Now, in contrast to beams, perhaps you might even recollect, but we also had this simplification in beams we said that along one dimension, they are much larger, the so-called slender beam theory, as compared to the other dimensions, so width and thickness in the cross section are much smaller than the length of the beam.

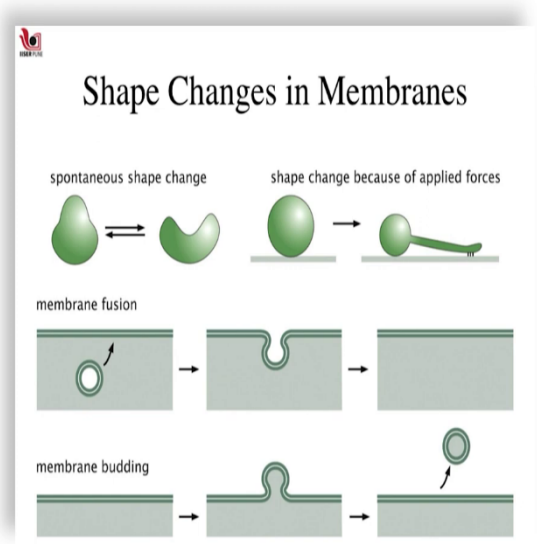
In a perhaps analogous manner, here the molecular component the lipids are a few nanometres, in size, two nanometers precise, as we had estimated and people have more accurately measured, whereas the membrane extends in this sort of a slab like representation of a cartoon lipid bilayer can be micrometres in scale, which is three orders of magnitude larger. And you can find them others, planar lipid bilayers, or curved by layers in terms of either vesicles or even cells.

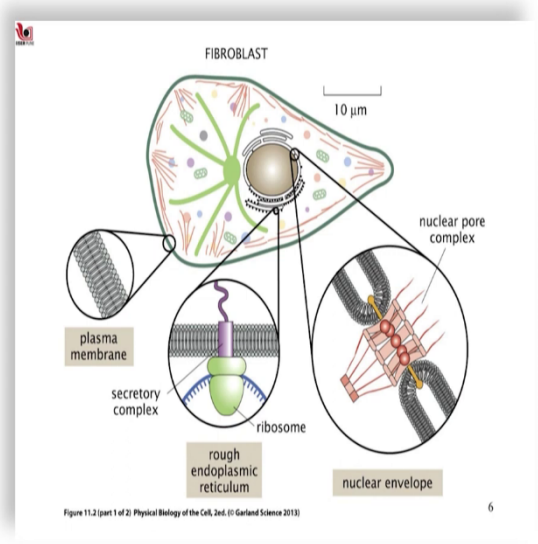
(Refer Slide Time: 01:52)



Now, it is apparent that the lipids themselves are dynamic in the membrane. So diffusion of lipids in the layer itself can take place as well, as it can flip between the bilayers, both spontaneously as well as driven by enzymes called flippases, whose function is kind of obvious from the name, which encourages moving from one layer to the other. In addition, memory proteins also diffuse in the membrane, and these also are capable of, perhaps in some cases being channels, and providing potential differences and creating more functional diversification.

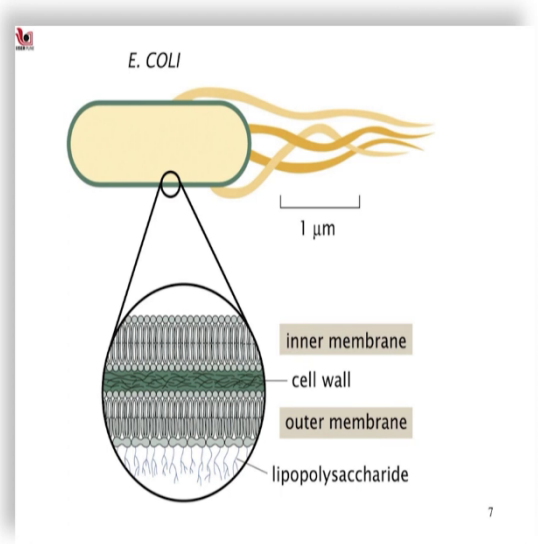
(Refer Slide Time: 02:35)





So, along with all this we also know that membranes undergo shift changes, they can be spontaneous changes or those due to applied forces, which means we need to understand the mechanics in order to understand how membranes function in vivo, both as the plasma membrane, as endoplasmic reticulum or you may say organelle membrane or even the nuclear membrane in eukaryotes, where they form these kind of double membrane structures, joined or sealed, you may say, by nuclear power complexes, and rigged with entry deregulated.

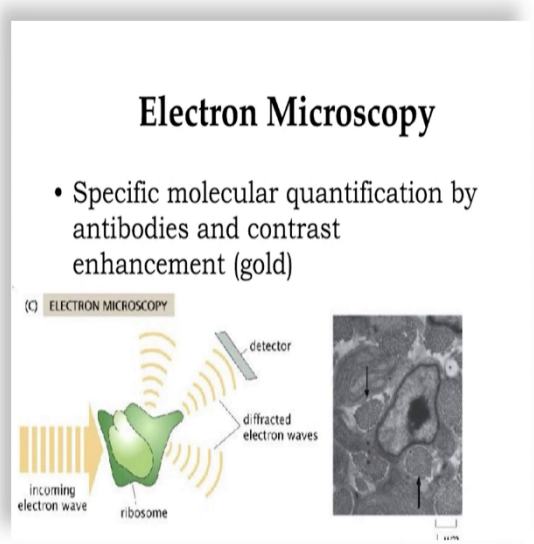
(Refer Slide Time: 03:08)

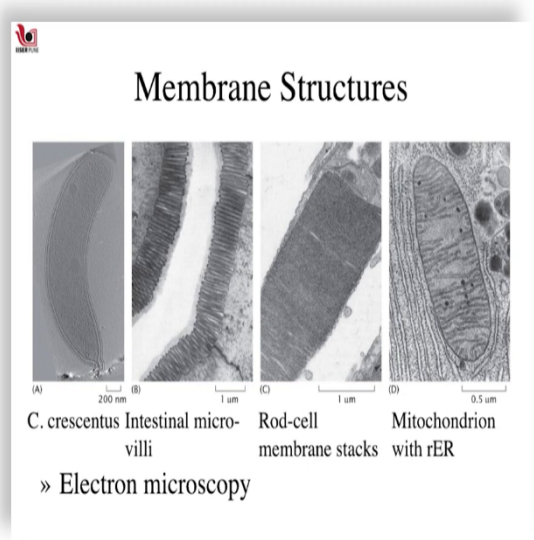


In fact, in our classic model organism of *E.coli*, you can observe that we have both limit by layer membrane, and an outer membrane separated by a cell wall usually peptidoglycan, and a lipopolysaccharide layer on the very outside. So, in some senses this double membrane is

also probably what the cell walls allows the gram staining to show up as a way to distinguish between this type and gram-positive bacteria, but this is for a different class, and I encourage those of you taking microbiology to ask this kind of simple question with respect to membrane biophysics, what are the functional differences that perhaps may be attributed to the membrane properties?

(Refer Slide Time: 03:52)

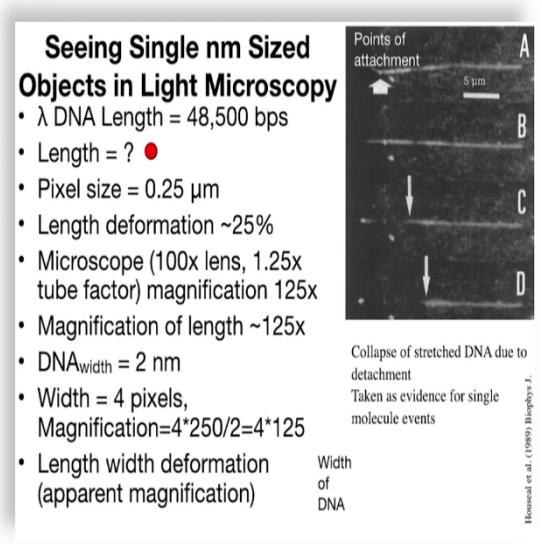




At any rate, electron microscopy has been a very widely used method to study membranes, but the method of impinging electron waves, and detecting the diffracted material, inherently tends to be static. Having said that beautiful membrane structures have been seen as is observed here with Caulobacter crescent is the bacterium intestinal, microvilli, rod cell

membrane stacks, in neurons and mitochondrial membranes with rough endoplasmic reticulum.

(Refer Slide Time: 04:25)



So, in a way, if you want to look at dynamics, light microscopy has been the favourite tool for the longest time, but the question about membranes has always been the problem that with the extent the thickness of single molecules being about two nanometres, how do you see them? And this sort of leads to this whole question about how you see the limited sized objects in light microscopy, when as we have said perhaps earlier, the resolution limit of light microscopy is indeed in the nanometre scale, and this is coming back to some of the lectures that Adish gave you with relation to techniques of single molecular imaging.

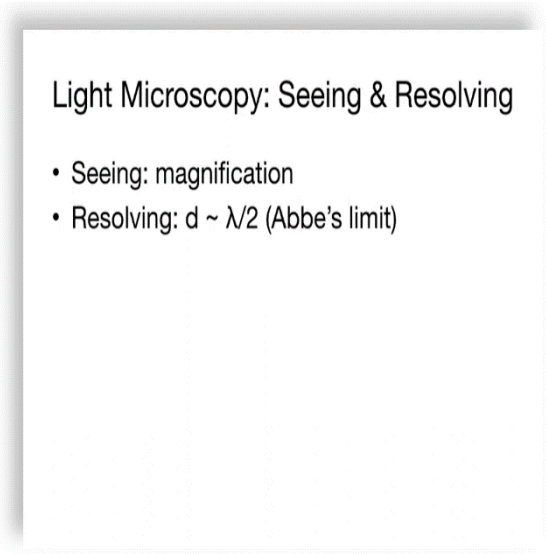
And I will leave this for our discussion on Thursday about DNA of length 48,500 base pairs that you can see in this image here, which was imaged already in the 19, late 1980s, 1989 using a fluorescent die, where you could see a single data molecule. Now, I hope you realize this or I want to emphasize this perhaps, the difference between seeing and resolving, and I want to ask you this question in our Thursday meeting, why do you think they can see?

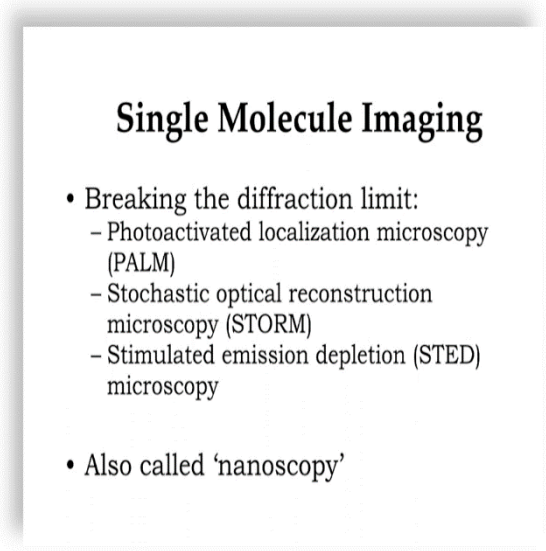
Because the fact is that given a certain length in base pairs, what is the length in meter units? This is the first question, I am going to ask you. What are the pixel sizes that we are observing here, this is indeed in this case of the image, it is 0.25 micrometres. What is the pixel? Well a, digital image like you are looking at here, is made up of integral elements or discrete elements that are elements of the picture, hence picture elements pixels, c, e, l, became x here, it is much cooler, making that x sounds nicer.

And, when we observe this in microscopy, the length, the absolute length, and the length deformation we observe is approximately 25 percent, and you can back calculate this from the fact that the microscope is using 100x lens with a 1.252 factor and a magnification, therefore 125x, back calculating the length u, and the width, you should bet get an idea of what is the width that we can actually see versus the width that we can, we expect from theory.

This length with deformation is indeed the apparent magnification. I am going to leave this for our discussion in terms of what you make sense, what sense you can make of this, and what are the answers to some of these questions, about why we can see single molecules of DNA given how narrow they are with the two diameters width.

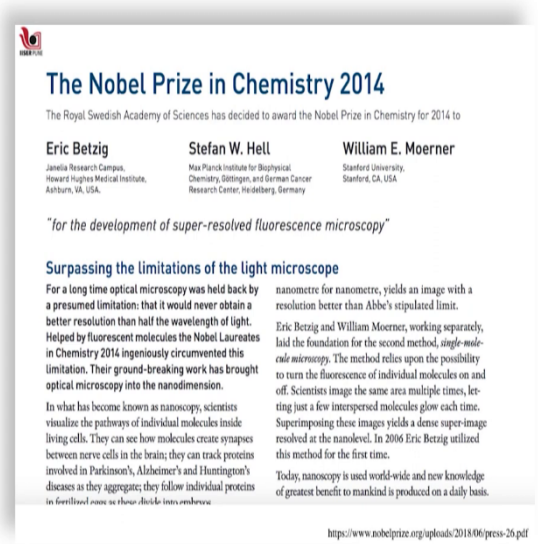
(Refer Slide Time: 07:02)





So, this is indeed seeing magnification resolving as separating two points, the resolution limit or abs limit as the Adish had pointed out is approximated by λ/2. λ is the emission, is the wavelength of light being used. And, this basically brought us to single molecule imaging which we talked about and three methods, I am listing here PALM, STORM and STED are some things that I shared papers with you for reading about nanoscopy.

(Refer Slide Time: 07:32)



The Nobel Prize in Chemistry 2014

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry for 2014 to

Eric Betzig
Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA.

Stefan W. Hell
Max Planck Institute for Biophysical Chemistry, Göttingen, and German Cancer Research Center, Heidelberg, Germany

William E. Moerner
Stanford University, Stanford, CA, USA

"for the development of super-resolved fluorescence microscopy"

Surpassing the limitations of the light microscope

For a long time optical microscopy was held back by a presumed limitation: that it would never obtain a better resolution than half the wavelength of light. Helped by fluorescent molecules the Nobel Laureates in Chemistry 2014 ingeniously circumvented this limitation. Their ground-breaking work has brought optical microscopy into the nanodimension.

In what has become known as nanoscopy, scientists visualize the pathways of individual molecules inside living cells. They can see how molecules create synapses between nerve cells in the brain; they can track proteins involved in Parkinson's, Alzheimer's and Huntington's diseases as they aggregate; they follow individual proteins in fertilized eggs as these divide into embryos.

nanometre for nanometre, yields an image with a resolution better than Abbe's stipulated limit.

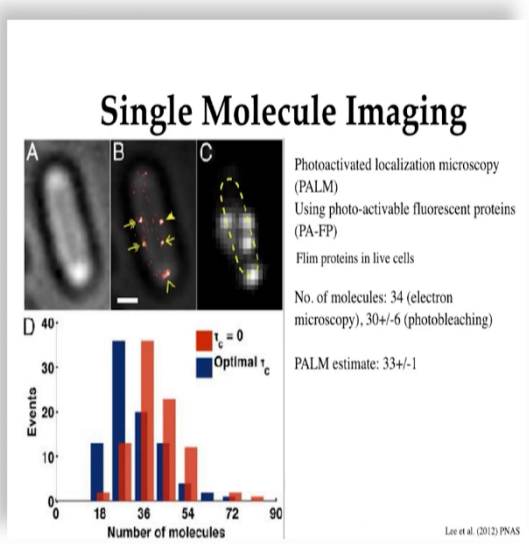
Eric Betzig and William Moerner, working separately, laid the foundation for the second method, *single-molecule microscopy*. The method relies upon the possibility to turn the fluorescence of individual molecules on and off. Scientists image the same area multiple times, letting just a few interspersed molecules glow each time. Superimposing these images yields a dense super-image resolved at the nanolevel. In 2006 Eric Betzig utilized this method for the first time.

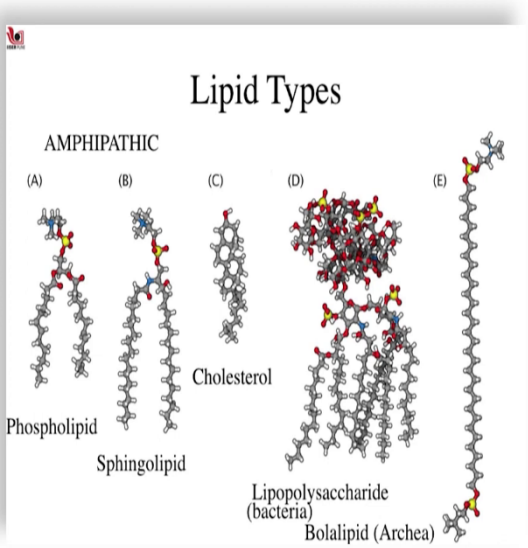
Today, nanoscopy is used world-wide and new knowledge of greatest benefit to mankind is produced on a daily basis.

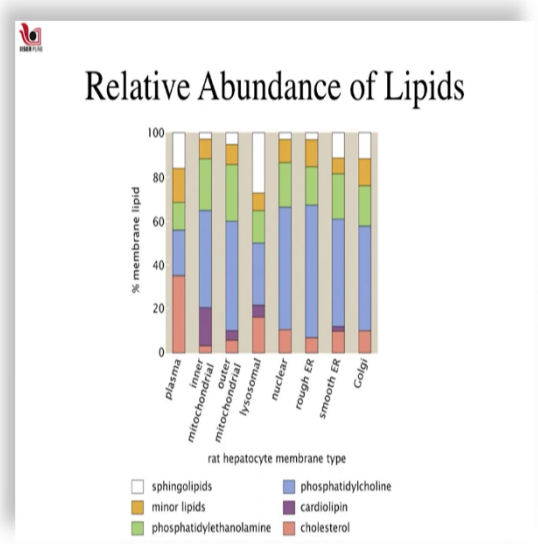
https://www.nobelprize.org/uploads/2018/06/press-26.pdf

And, I would just like to remind you about the interdisciplinary nature of biophysics in general, because I would call these methods biophysics, but the Nobel prizes were given for chemistry. In fact Stefan Hell is a biophysical chemist, he is at the Max Plank for biophysical chemistry in goettingen and the university of Heidelberg. Bill Berner is a chemist, he developed a lot of the dyes, and Eric Betzig is an engineer and who was encouraged by a cell biologist namely Jennifer Lebankor Schwartz to come back to research and try and help develop photoactivation light microscope.

(Refer Slide Time: 08:09)



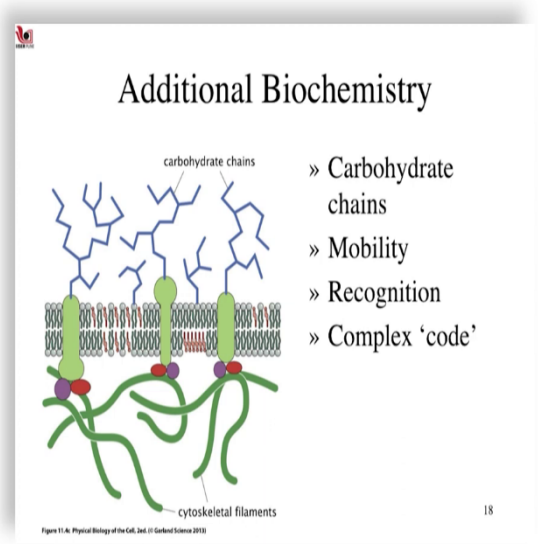




So, given how much we have talked about seeing molecule imaging, I am going to skip over these slides, and basically jump straight back to our lipids where we are concerned. To the kinds of lipids, so phospholipids, lipids, cholesterol level, polysaccharides, bacteria, biologists, polar lipids, I am sorry, in archaea are some of the types of lipids, that you are likely to expect from a biochemical perspective, that will likely be amphipathic.

And, you can look at relative abundances of lipids based on the graphs over here. These are just simply indicative of the fact that these lipid types vary between even organelles in the same cell type, red hepatocytes, right levels.

(Refer Slide Time: 08:51)

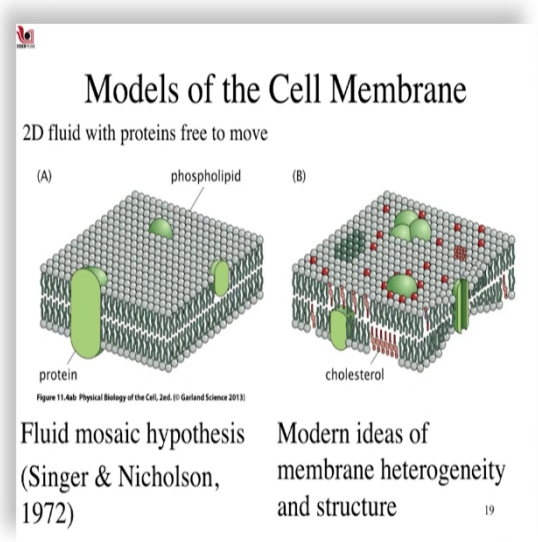


Additionally, along with the lipid component and the proteins that are in them, there are also carbohydrate chains, and these form a sort of additional code that further complicate the bio,

biochemistry of lipids. They are also capable of modifying mobility, and the recognition forming a very complex code that membranes can encode, and in a way you can argue the DNA code is kind of simple, four letters, the RNA code is related to the DNA code, but it encodes for the protein recognition, so it is a bit more complex, it has triplets, codons, Khorana and Kornberg discovered.

The protein code itself is a little more complex, and now when we come to the lipid code, it is very complex, and I think if you are really contemplating a career in biochemistry, and cell recognition, and molecular biology, I would say this is the future to work on. But do not take my word for it, go talk to your biochemistry faculty, and they will probably confirm some of this. Thomas Pucadyil is an authority in the field, and I really encourage you, if you are excited by this, go to it. Anyway, advertisements aside.

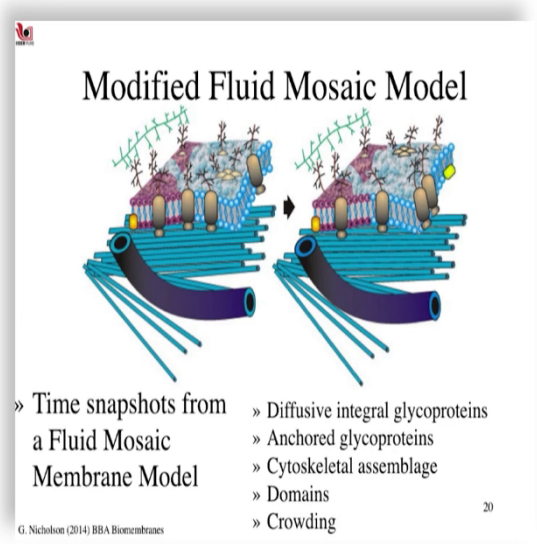
(Refer Slide Time: 10:00)



So, models for the cell membrane are classic. And I think all of you have gone through school probably, or college, university, hearing about the fluid mosaic hypothesis saying, this is attributed to Singer & Nicholson, 1972, even before I was born, but I did read about it, or maybe that is why I read about it. Modern membranes, membrane models or ideas of membranes, involve an idea of heterogeneity and structure.

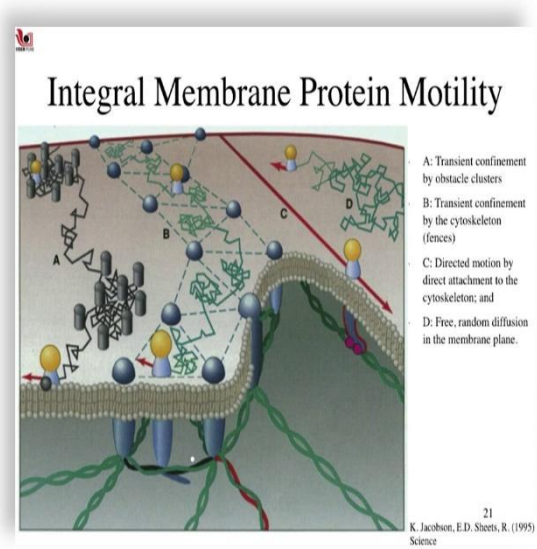
So it is not just a sea of lipids in which some proteins are bobbing around, like a little boat, chota nauka, in a, in a river of, in an ocean of lipids, but also that the lipids themselves are diverse, that the proteins may be integral membrane proteins, only in one layer of the bilayer, there may be structure to the lipids, and so on and so forth.

(Refer Slide Time: 10:52)



And this has led people to propose a so-called modified fluid mosaic model, and these are supposed to be time snapshots, the left and right images, which represent the role of diffusive integra, integral diffusive glycoproteins, so proteins with the modifications, excuse me, of sugars, cytoskeletal assemblages, the tubes that you see below, and the blue lines indicating basically microtubules and actin. And the idea that there are domains that, that are special in the lipid due to enrichment of either cholesterol or rich or poor regions.

(Refer Slide Time: 11:33)



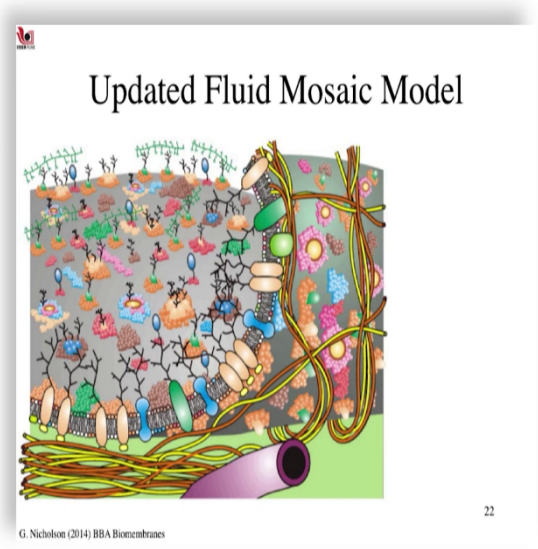
They need integral membrane protein mobility itself, meaning to say membrane proteins that span both bilayers have been considered to be at least classified into the following four kinds. Transient confinement by obstacle clustering, transient contaminants cytoskeleton offenses,

directed motion by direct attachment to the cytoskeleton, and free random diffusion in the membrane plane.

And as some of the work that we have done in our lab has shown in the past the very nature of these mobilities, can determine the overall statistics of how often a membrane protein is found together with another, in form of either dimer or trimmer or tetramer and so on. And these in turn, as in the case of cancer, regulatory proteins like receptor tyrosine, kinases like EGFR, can also regulate cell function.

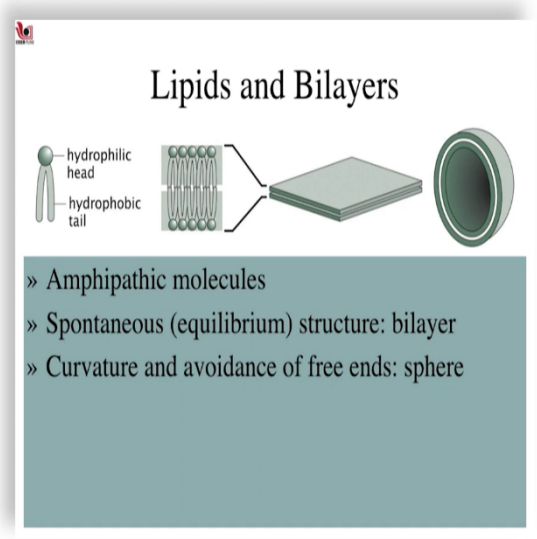
So at one level this membrane protein mobilities of very exciting academic question how things are, but there is a lot of relevance to disease also. And I really encourage you, if you are interested in looking more into Ken Jacobs and Dale's works, because they are sort of authorities in the field, and these offer a nice little summary of it.

(Refer Slide Time: 12:51)



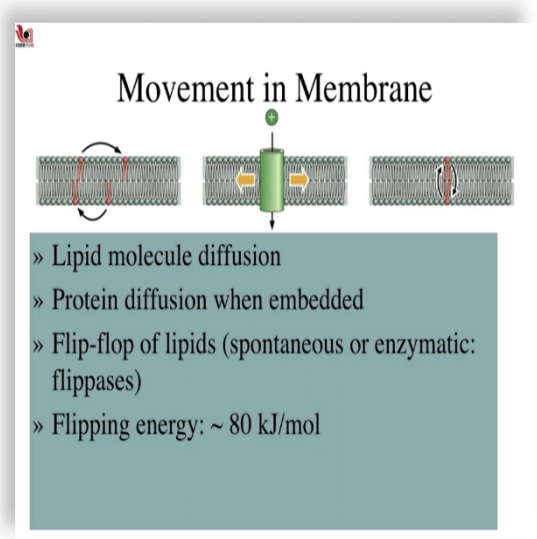
So, the updated fluid mosaic model looks a bit messier, there are little islands, and there are these tree-like structures, which are the glycoproteins, and the glycoproteins themselves are mostly integral, some of them are only in one bilayer, and there is this whole wiring diagram of the wires of actin, underlying it the signalling proteins and then ubiquitous microtubules.

(Refer Slide Time: 13:12)



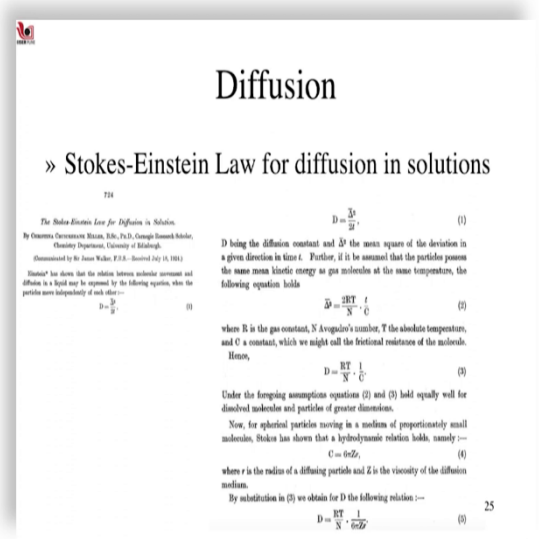
So, coming back to our lipids and by layers, we do think that they have amphipathic molecules, they are spontaneous equilibrium structures forming bilayers. Curvature avoidance of free ends leads to sphere formation.

(Refer Slide Time: 13:25)



Lipid molecule itself diffuses, protein diffusion, when embedded, is driven by thermal energy. Flip-flop of lipids is spontaneous or enzymatic, and flipping energy is up to 10 kilojoules per mol.

(Refer Slide Time: 13:49)

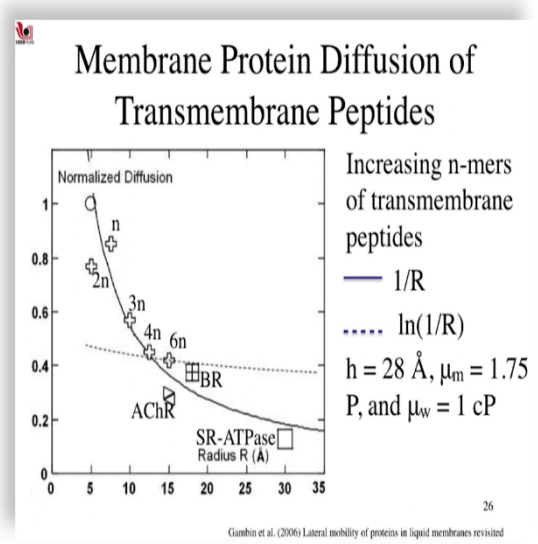


So when we talk about diffusion, we always ask so what drives diffusion? And some of you maybe have taken a course in physics that told you this. But suffice to say that the Stokes-Einstein law for diffusion and solutions, cleanly defines for us what happens in solution in one, two or three dimensions, and three dimensions is what we would imagine is the largest degree of freedom.

And this little review, essentially, summarizes the relationship, the Stokes-Einstein relationship, that D is the diffusion coefficient, and Δs is the mean square displacement of the particle in a certain time t, and D is equal to Δs by 2 D. And you can also write this in terms of the Stokes' drag coefficient which is the equation 4, where for spherical object, the drag coefficient is $6\pi\eta R$.

$\eta$is represented here, I think by Z, and indeed this is your dynamic viscosity that we talked about last week, R is the radius of the spherical object diffusing, and 6 and π are constants. So, in such a case, by substitution, the rate of the so-called diffusion coefficient is k_BT, that is RT upon N, is just simply k_BT, upon $6\pi\eta R$. This is the famous, celebrated, in my view, celebrated Stokes-Einstein relation.

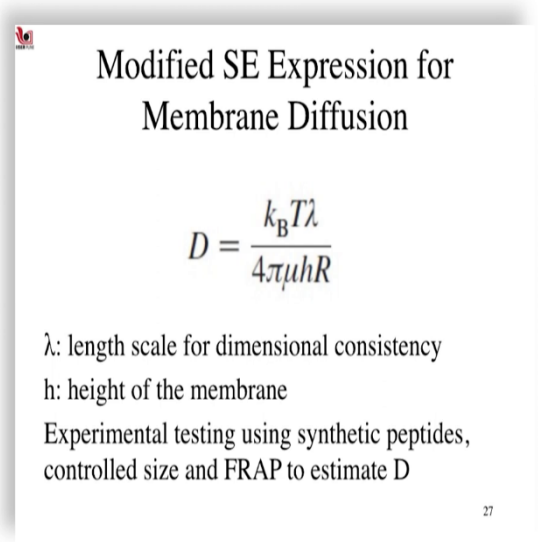
(Refer Slide Time: 15:15)



Now, funnily enough, when you look at membranes, the size dependence on the x-axis, you are looking at radius and angstroms, so if you take one by 10 of that it is nanometres, and different proteins, then the 1 by r dependence, so remember, $6\pi\eta R$ is in the denominator, so everything else if, you assume, is constant, k_BT is constant, is $6\pi\eta$constant, assuming the viscosity is constant. Then, the dependence of diffusion equation should be about 1 by R.

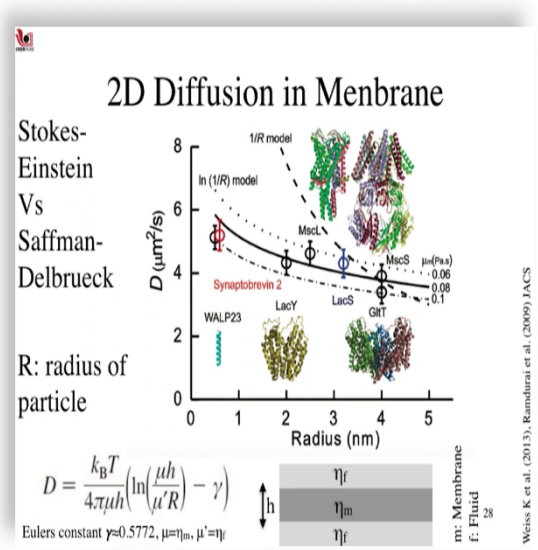
In such a case, in this data, what you observe is that proteins of increasing sizes of n-mers of transmembrane peptides, appeared in this experimental data by Gambin et al, to indeed, in lateral mobility show a one-by-one dependence. That was great, I mean that that suggested that basically Stoke-Einstein's relation works for diffusion coefficients.

(Refer Slide Time: 16:13)



But new data appears to suggest that we need a modified expression for membrane division, which includes the height of the membrane, and a factor lambda, and the factor λ is the length scale for dimensional consistency, and h is the height. In such a case, this shows that it is capable of better explaining experimental data which were made using synthetic peptides for control sizes and using photo fluorescence recovery, after photo bleaching of frap to estimate the division coefficient.

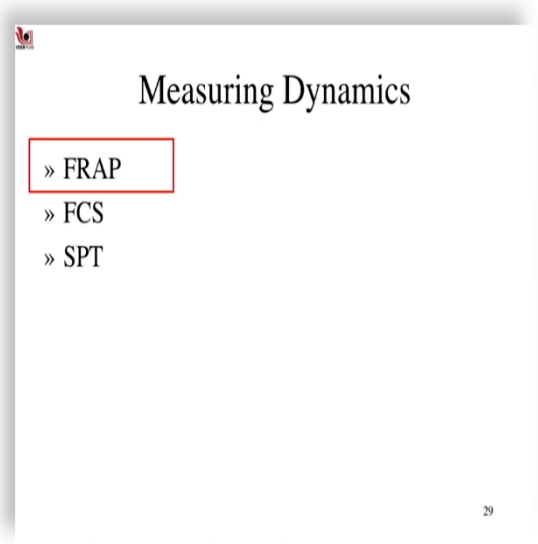
(Refer Slide Time: 16:47)



So, what you are looking at here is the comparison between Stokes-Einstein relation for diffusion estimation to the Saffman Delbruck estimation of D, which is in the slightly more complex forms $k_B \frac{T}{4}\pi\mu h$, where mu is basically the viscosity of the membrane, internal viscosity of membrane, h is the height, and then in the brackets is a natural log term with $\mu\frac{h}{\mu}'R$, where μ'mu prime is the fluid viscosity.

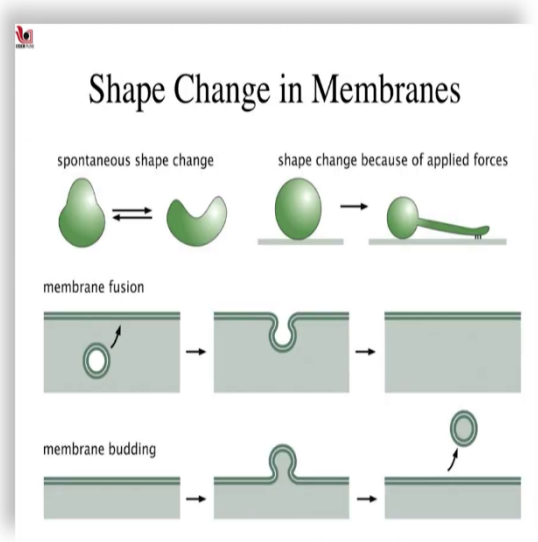
So you see this in this schematic here on the right hand side lower right, where membrane viscosity is the η_m, and fluid viscosity is the fluid above and below it. In other words, the cytoplasm and the outside. So we are assuming those two are the same here, sort of water like. In such a case, the 1 by r model overestimates the diffusion question for small molecules, which are seen to follow a slower dependence. In other words, the diffusion coefficient is not as high as expected from Stokes-Einstein theory.

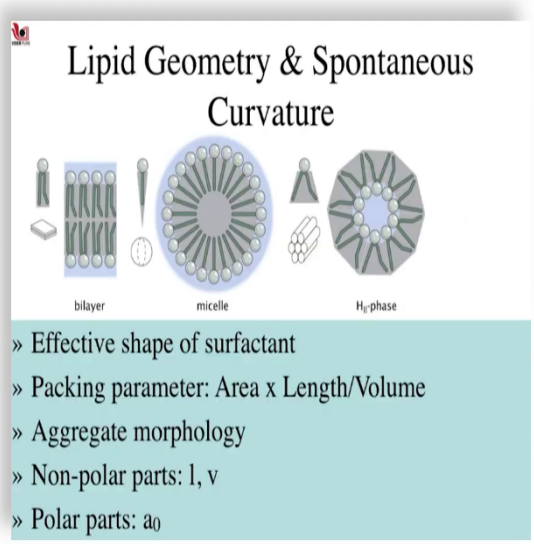
(Refer Slide Time: 17:58)



And, so dynamics and membranes can be measured using fluorescence, F words, basically fluorescence recovery after photo bleaching, fluorescence correlation spectroscopy, single molecule, single particle tracking. And we are going to be hearing more about FCS from a guest lecture in middle of this month, who is an expert at developing, in fact FCS methods and has some real innovations to his name.

(Refer Slide Time: 18:29)





So, indeed the geometry mechanics of membranes, should probably be determined by the constituent elements that make them up. So, for the next segment, I am going to talk to you about lipid geometry and spontaneous curvature, and how properties such as the packing parameter which combine area length, area and relative length to volume ratio, and how they determine aggregate morphology using some simple geometric calculations.