Physics of Biological Systems
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Lecture – 05
System Variability and Spatial Scales

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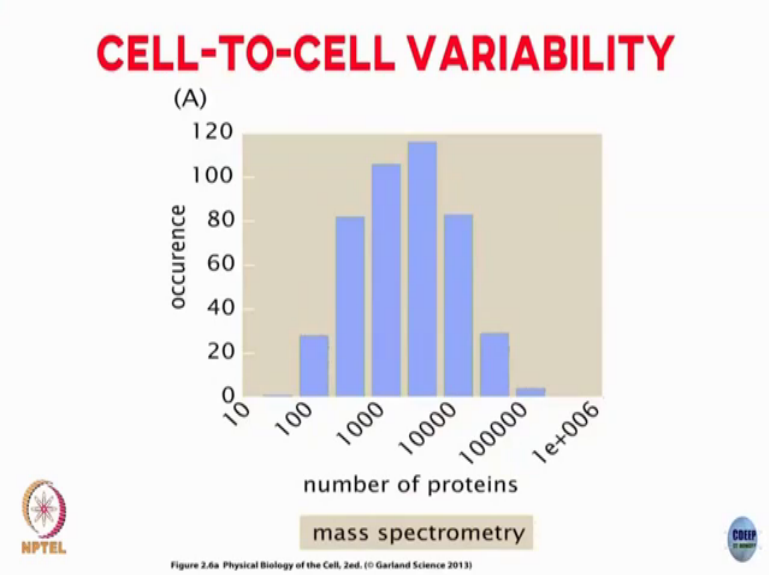

No number is sort of set in stone, and you can have really really wide fluctuations depending on what quantity you are looking at all right. What could these, so if I took two cells let us say two E-coli cells cell a and cell b, and I see in one there is some 10 to the power of 6 proteins, in another there may be a 1000 proteins ok. What sort of processes could give rise to this variability any guesses? It is the number of proteins I can go back and check Rob Phillips and see what the system was.

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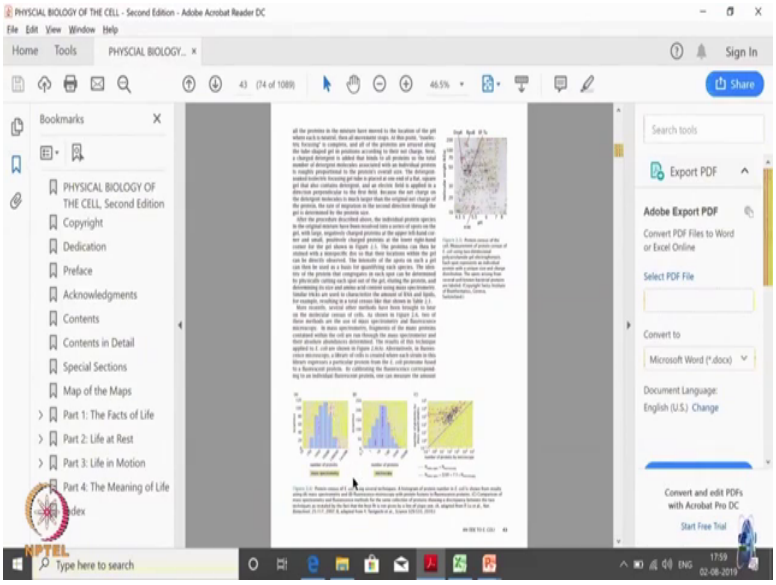

Let us see which figure was this, this is figure 2.6. This is protein census of E-coli, yes it is Nature Biotechnology, 2007. I will put that reference in sorry you cannot see material. This is mass spectroscopy and fluorescence microscopy ok. Say I have one simple the answer is of course complicated, but I will just show one possible cause of this variation which is let us say cell division ok.

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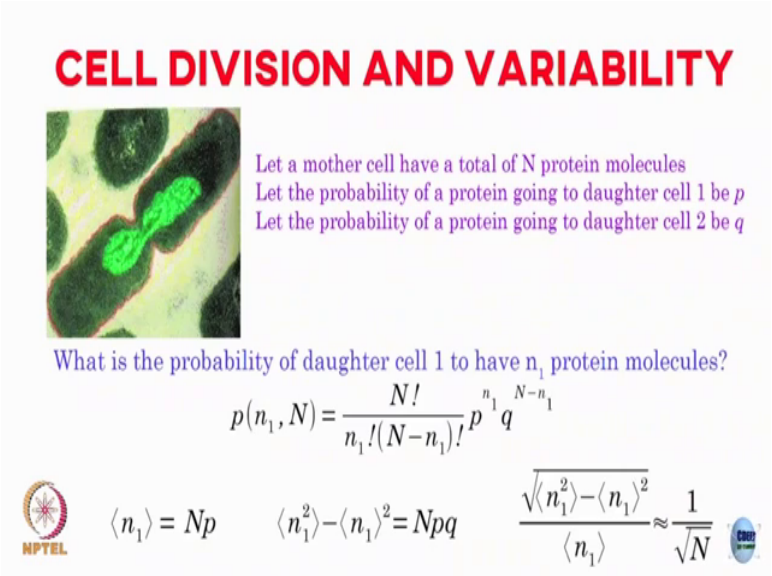

So, when you have a cell dividing into two daughter cells, each daughter cell will get some copies of the proteins of the mother cell right. So, let me do a very simple very naive calculation. So, I say that a mother cell has some total N protein molecules ok. And the probability of that protein molecule was, one protein molecule going to daughter cell 1 is p, and the probability of a protein going to daughter cell 2 is q. And I could ask that well what is the probability that daughter cell 1 has some n 1 number of protein molecules.

What would be that probability what distribution is that the binomial distribution right. So, with probability p you sent n 1 number of molecules to its daughter 1 therefore, with probability q, you send N minus capital N minus n 1 molecules to daughter cell 2 this is N c n 1 because that is the probability the daughter cell 1 has n 1 protein molecules. What would be

the mean number of protein molecules that this daughter cell would have daughter cell 1, let us say N p, what would be the standard deviation of the variance N p q right.

So, if I looked at fluctuations, if I compare the fluctuations to the mean I get that the fluctuations are tamped down as 1 over square root of N right with a very standard result in binomial (Refer Time: 03:06). So, the variability can be very large depending on how large your n is where n is extremely large and the fluctuations are fluctuations by this process through cell division would be small.

However, if the number of copies of the protein of some protein is small it has 100 copies or a 1000 copies, you can get very large variation simply due to cell division itself.

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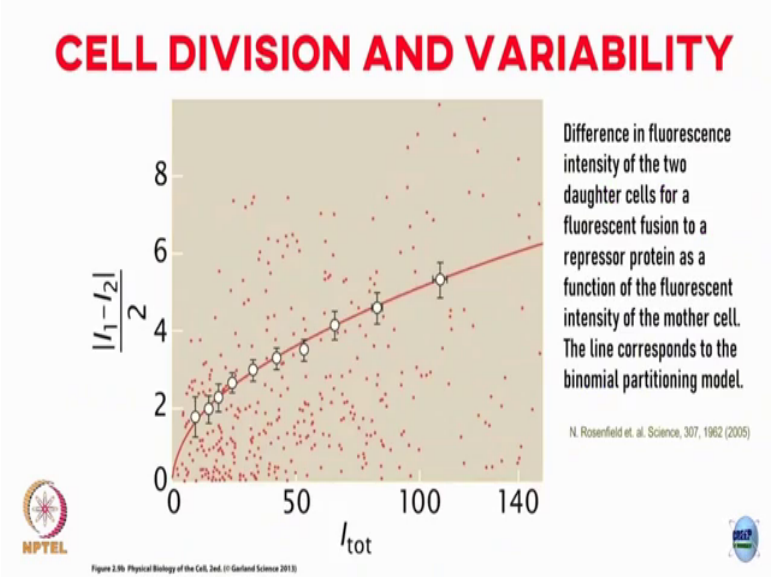

So, here is one experiment this is one particular protein which has a fluorescent tag fused to it, and then you measure the difference in fluorescent intensity between the two daughter cells as a function of the fluorescent intensity of the mother cell ok. So, you have one cell which divides into two.

The mother cell has these one particular protein that you are interested in, and you fuse a fluorescent tag to it the mother cell divides into two daughter cells, you measure the intensity of the mother cell and the intensity of the two daughter cells, and you plot this difference in intensity as a function of the intensity of the mother cell. So, these are all data points. So, it is all over the place.

But if you did a mean sort of these points represent the mean, so if a mother cell has intensity of 50, the difference in the daughter cell intensity is roughly comes out to somewhere 2.5, 2.6 something like that. The solid line is this binomial partitioning that we discussed ok. So, of course, there is a lot of variability, but if you were just looking at the mean properties, the binomial partitioning looks to be not too bad a model ok. Would this always be true? No, there could be other processes going on that leads to this variability it.

So, happens that binomial partitioning is one of the factors that play a role. It is not the answer, it is one part of the answer ok. It so happens that this for this particular protein the variability comes because of this division and that is why you get a reasonably nice agreement with the binomial partitioning model. So, the message is this that at least one of the messages is this that even though it looks the problem looks complicated, you could try very simple models, you could try very sort of naïve modeling approaches.

They may be enough to answer certain, it will not; it will not work for any protein and so on, but at least for some cases even naive simplistic modelings might give you a reasonable fit to the experimental data after all binomial is something that we know from school probably. So, that is one source of variability.

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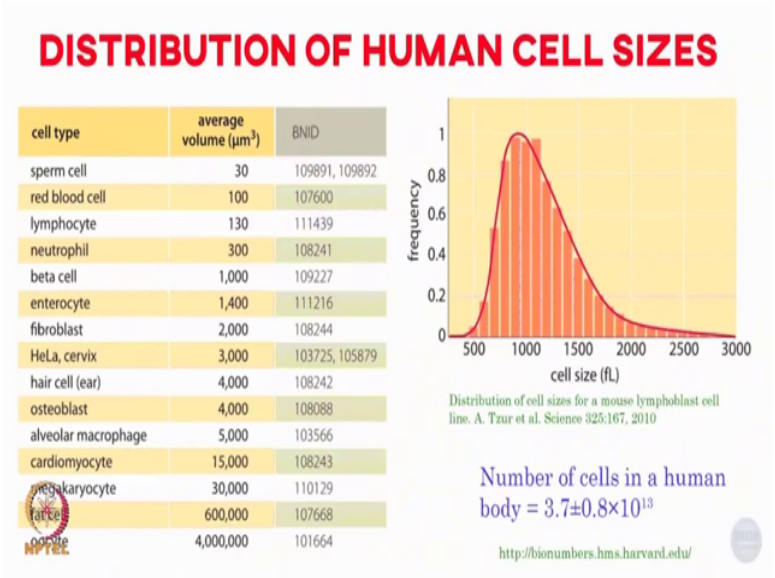

You can think about what are the sources of variability there could be all right. Before I end I just had this very nice curve which I found interesting. So, it is the distribution of human cell sizes again to so show sort of variability. So, these are different cell types sperm, red blood cell, lymphocyte, neutrophil and so on. You span a whole range of volumes, so the sperm cell is one of the smallest, it is 30 micron cubed.

The fat cell or an (Refer Time: 06:14) on the other end of the spectrum, it is an extremely huge cell. So, again you can plot a histogram of the cell sizes. Well, this is not human; this is mouse. I do not think there is one like this for humans, but this is for a mouse lymphoblast cell. And the total number of cells if you estimate for in a human body is around what is this, this is around 37 trillion, 37 trillion cells ok.

So, that is; so that is one part, so we or rather two parts. So, we talked about this crowding, we talked about this fluctuations or noise. Yes.

Student: Sir, how did the how did (Refer Time: 06:56)?

In a sense that let us say I have a cell, I have a single protein.

Student: (Refer Time: 07:10).

This cell will divide into two daughter cells. This protein could end up either here or there right with some probability. Now, you have many many proteins, each of them let us say half, let us say with equal probability. So, with half probability, it could end up here or it could end up there right.

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So, when you do it is like a coin toss right. So, if you are ok. So, if you are tossing 1000 coins, on an average you would of trend tend to get 500, but you could get 200 in one and 800 in the other that would cause a variability.

Student: But the histogram was according to the size right.

The histogram was.

Student: According to the size of the.

This histogram.

Student: The previous one, previous one.

One second.

Student: Yeah, ok.

This is the number of proteins. So, when you take an E-coli cell the point is that the number of proteins is not fixed right in some cells you could have 10 to the power 6, and you take another E-coli cell maybe you have 10 to the power of 5 and so on.

So, then depending on how it divides or like you said cell division is not the only way it is not even a probably the most important reason of this fluctuation, it is just one case where I can do a very simple modeling and explain try to explain some of the data all right. So, let us, 6, ok.

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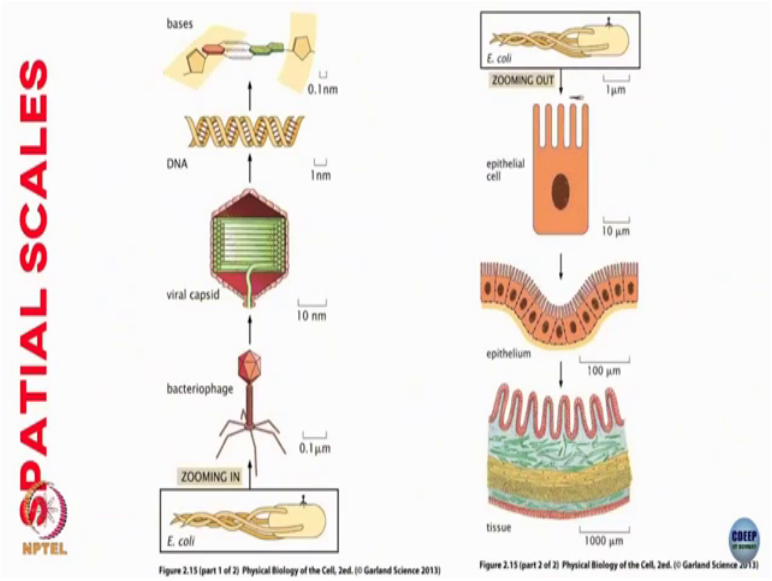

So, now that I have done at least a little bit of these two points let me move on and talk a little bit about what sort of spatial scales we have talking about. So, last class we talked about these DNA bases and that was around each base for is around 0.33 nanometers right. You can move up scales for example, a viral capsid was roughly around 10 nanometers, bacteria phase is around 0.1 microns. If you move up another order of magnitude the e-coli is around 1 microns right. So, here is the bacteria feed sitting on top of the E-coli.

You can grow go up even more so for example, an epithelial cell or may standard human cell, the number we take is roughly 10 microns. If you take the epithelium as a whole that is around 100 microns, tissues you can go up to 1000s of microns, and of course, then you go up to organisms so you can move up to like (Refer Time: 09:31) longs.

So, this is whole, so if you wanted to ask well how exactly does this tissue work how do the chemical processes the biological processes in this tissue work. And if you were looking for a really really comprehensive answer, you would need to go back you would need to figure out processes that were happening at all of these skills which is very difficult which is why we generally we do not ask such a broad question like this.

We restrict ourselves to one sort of scale and because the physics at each of these scales are very different the type of modeling that you would do is very different. We try to build put them together as much as you can, but spanning these wide magnitudal spatial scales from nanometers to meters is really really difficult task. See this is one of the reasons, but next biology so complicated.

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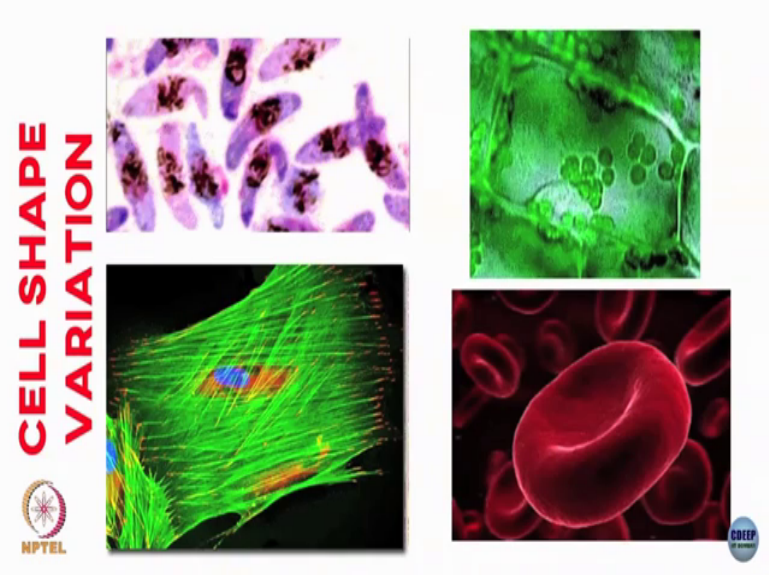

So, it is another thing, so this shows variations in cell shapes. This over here anyone knows what this is this is a pretty this is plasmodium falciparum. So, it is a single celled protozoan the malaria protozoan. This over here is a plant cell; this over here is an animal cell, and this is a red blood cell. So, you will see a variation in sort of shapes, a plant cell is very sort of rigid, it has a cell wall. Animal cells are sort of fluid, they do not have a rigid cell wall.

These things over here are the chloroplasts so I think. And here you see this very long objects which are the cytoskeleton filaments, the microtubules, and at the ends the actin filaments which again we will talk about. A red blood cell is slightly special in that it does not have at least a mammalian red blood cell does not have a nucleus and that is sort of allows it to squeeze through very narrow capillary species, but most cells will have most animals all and other animal cells will have a nucleus.

These are still fairly standard to sell shapes, and you can actually do some amount of mathematics to predict sort of types of cell shapes that you could have that is the next slide, but anyway.

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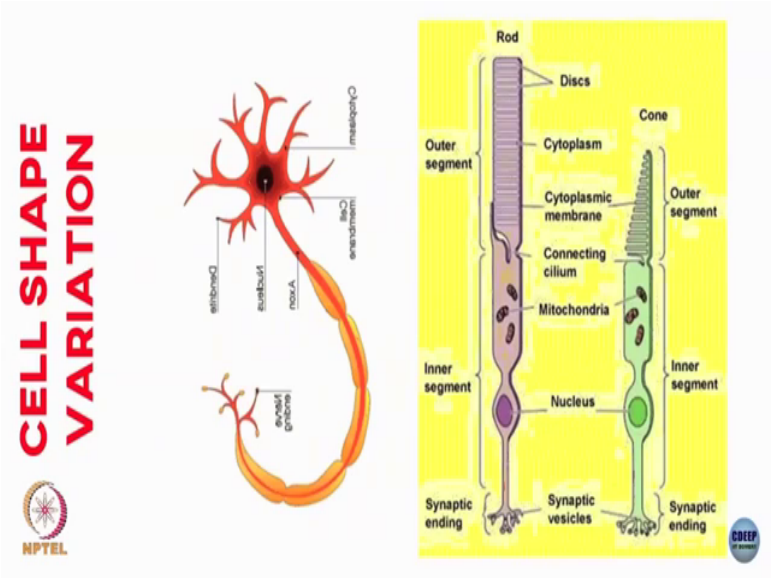

So, you could also have very weird looking cells. So, for example, this is a nerve cell. Here is the dendrites, this is the axon which is the cell body, and here are these nerve endings over here. These cells are the rod cells in the cone cells that allow you to see color and these are inside your eye, and these look extremely weird. As long as you are sort of restricting yourself to sort of basic shapes like these you could try to do sort of theoretical analysis of what sort of shapes would come up.

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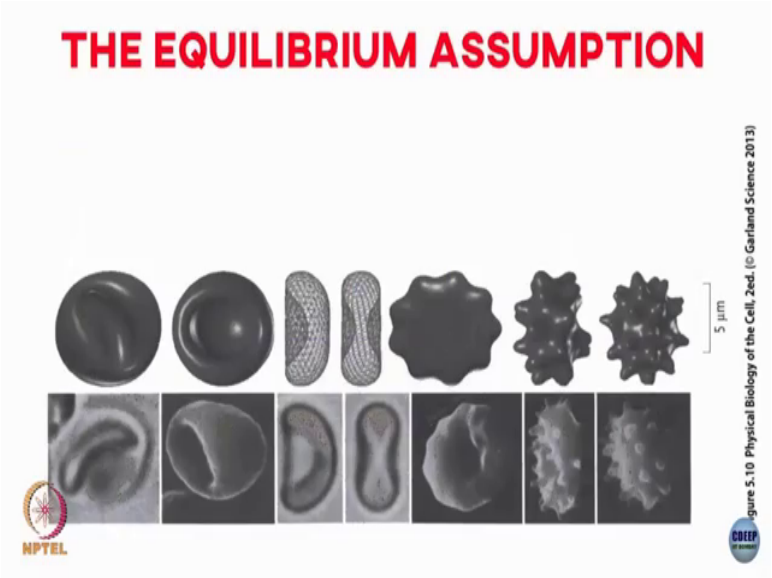

So, this is a result of a calculation where you sort of min, you write down a free energy or you write down an Hamiltonian for the sort of various terms that are involved that would go into determining the cell shapes, elastic energy, bending rigidity and so on. You minimize the free energy and is try to predict depending on what parameter values you have chosen, what sort of cell shapes you would get, the result of one such analysis, and it so it is used for red blood cells and predicts a whole variety of red blood cells. So, this is an equilibrium sort of calculation.

And even though we know that the body is not really at equilibrium, equilibrium calculations can often work out in different scenarios depending again on what sort of timescales and spatial scales you are talking about. This would not really work for this weird looking cells, but for standard cells this sort of calculation it is I think it is based on something called the

Helfrich, is based on the Helfrich free energy and again we will probably talk about it as we go along in the course all right, all right.

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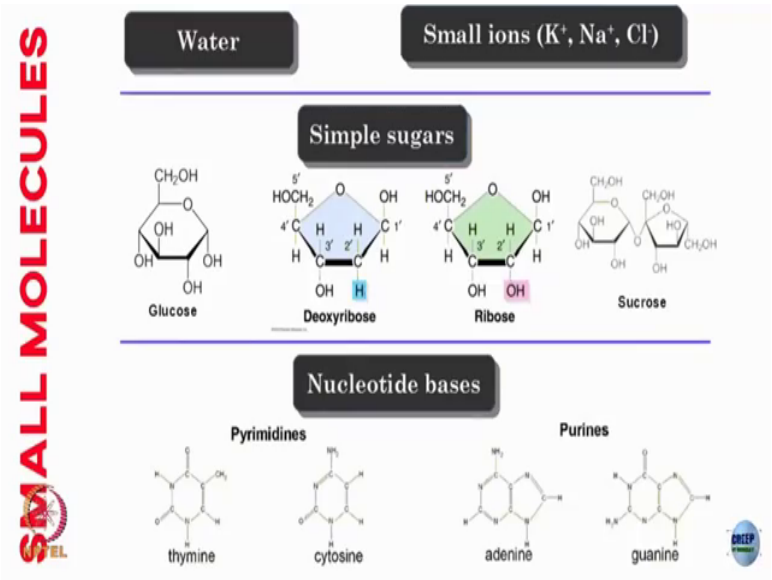

So, I think for the rest of the class let us try to just again get a little bit familiar with what, what all things are inside the cell. So, what makes up my cell. So, roughly nelson sort of classifies it into three things, small molecules, medium molecules, and macromolecules or large molecules. So, the small molecules are water of course, small ions potassium sodium and chlorine so on. Simple sugars so it could be sugars of 1 ring or 2.

So, glucose deoxyribose ribose have 1 rings sucrose is 2 rings it could be. This screen is not very good; at least the screen over there is somewhat better. You could have; you could have the 4 nucleotide bases thymine, cytosine, adenine, guanine. And again thymine and cytosine

are the pyrimidines which contains 1 ring, the adenine and guanine are the purines which contains 2 rings.

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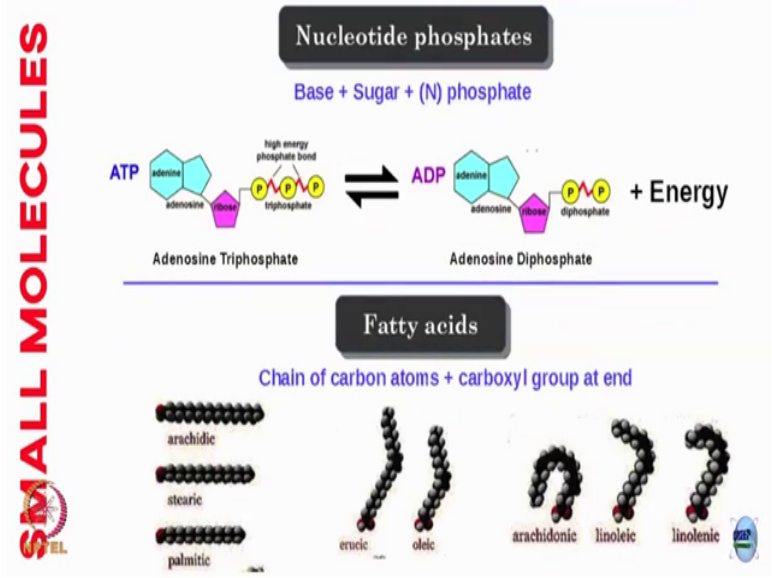

So, these are some small molecules, you could take one of these nucleotide bases thymine cytosine adenine guanine, add a sugar to it, one of this oxyribose deoxyribose and so on add a phosphate. So, if you take this adenine, you add 3 sugars, you add a ribose and you so you add one ribose and you add three phosphate bonds that gives you what is called adenosine triphosphate or ATP. You have heard of ATP that is like the energy currency of the cell, so that is where the cell stores its energy.

This ATP you can hydrolyze one of these phosphate bonds to give ADP - adenosine diphosphate where you have only two phosphate molecules plus it releases some energy. This energy the cell uses to perform whatever actions it needs to perform. Similarly, you could

have guanine triphosphate hydrolyzing to guanosine triphosphate hydrolyzing to guanosine diphosphate plus energy. So, this is the sort of energy currency of the cell ATP, GTP and so on.

So, you take a base a nucleotide base, you add a sugar, you add some number of phosphates and that is to some energy. Now, other sort of small molecule are these fatty acids which are chains of carbon atoms with a carboxyl group at the end, and then depending on how long this chains are you have different fatty acids, palmitic fatty acid, stearic, arachidic and so on ok.

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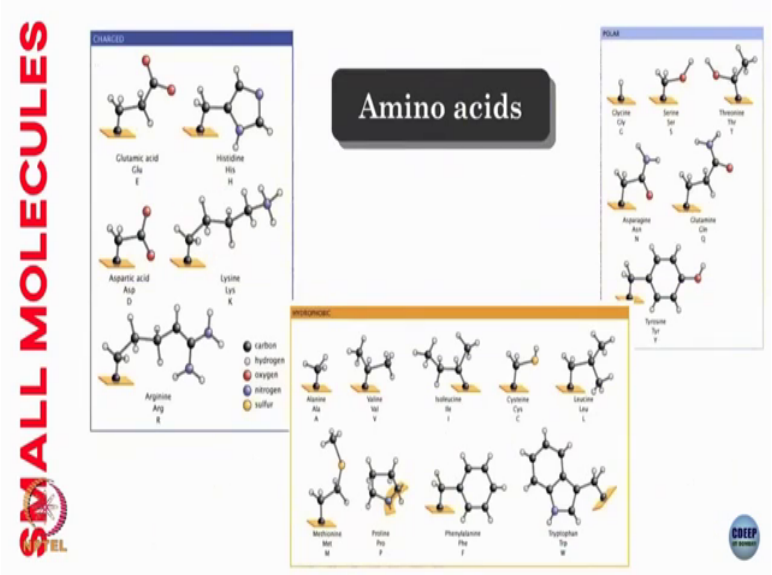

And the final category are what we already talked about these amino acids. The amino acids you could group into three classes, charged amino acids hydrophobic amino acids and polar amino acids. And depending on the whether they are charged or not whether they are

hydrophobic or they are hydrophilic it will sort of reflect on the secondary structure, how they assemble whether they want to stay in contact with water or whether they want to repel water and so on. So, these are roughly my small molecules.

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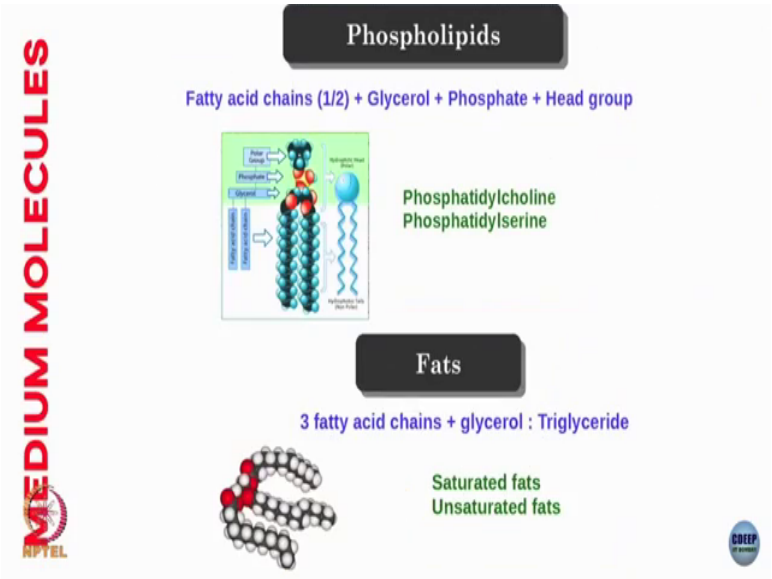

Then you move into medium range molecules. So, for example, one is phospholipids where you take a fatty acid chain which we talked about. You add a glycerol, a phosphate and a head group, and you get a phospholipid. So, the name sort of thin coats it in for example, if you have phosphatidylcholine, it means that the head group is choline; if you have phosphatidylserine, the head group is serine. And this is sort of a representation.

So, there here is your head, which is hydrophilic. Here are your fatty acid tails which are hydrophobic, and these phospholipids are found where these phospholipids are found in various membranes ok. So, they assemble the self assemble into sort of by layers.

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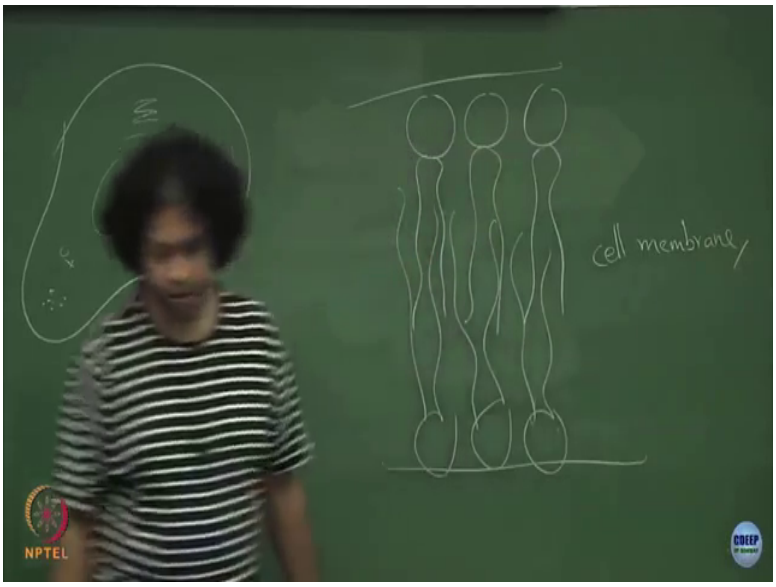

So, if you have this sort of a phospholipid, here is your head group which is hydrophobic. Here are your fatty acid tails and you put many of them together. They will try to expose the hydrophobic heads and protect their sorry they try to expose the hydrophilic heads and try to protect the hydrophobic tails. So, you get a bilayer like this. So, this is what the cell membrane is sort of made up of.

Then another category of medium molecules is what we would call fats. So, there you take three fatty acid chains, you add glycerol that is what is called triglyceride. Depending on whether you have double bonds or single bonds you could have saturated fats and unsaturated fats, you should eat one you should not eat the other all right. What else?

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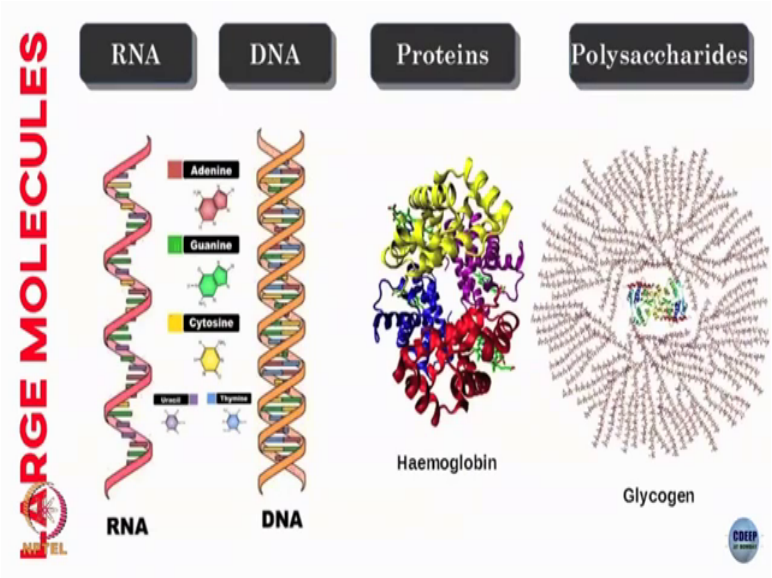

Then you have these large molecules, so the macromolecules. So, for example, RNA, you have DNA, you could have proteins; you could have polysaccharides like glycogen. So, here again is the difference in the bases between RNA and DNA is that in RNA the thymine is replaced with yourself like we talked last class ok. So, these are one category of large molecules, you could also have macromolecular assemblies.

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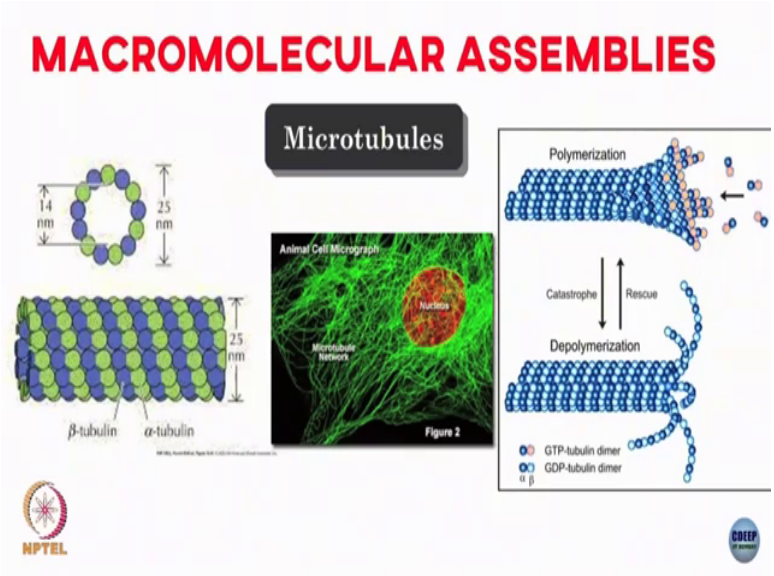

So, for example, if you remember this picture of the cell, you have this very long sort of objects which span all of the cell. So, this is what is called a microtubule. And the microtubule is made up of a very basic protein unit which is called dimer, it is called the tubulin dimer, which contains alpha tubulin, and a beta tubulin.

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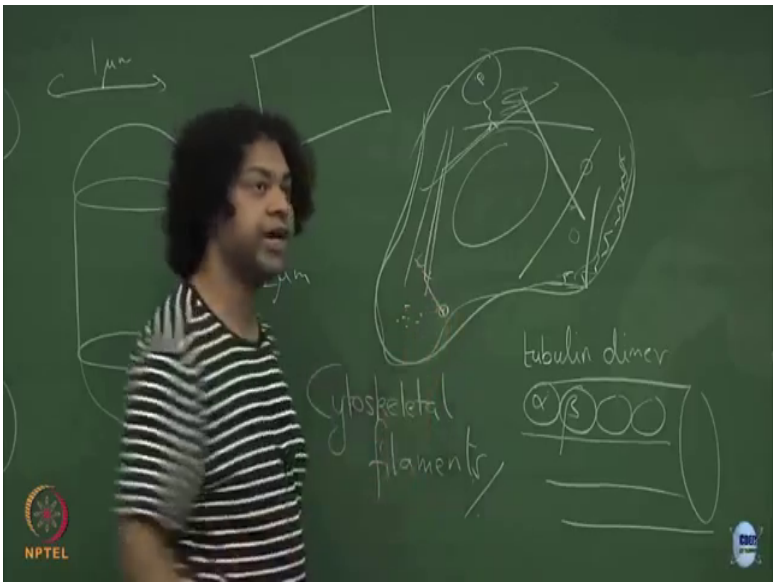

It contains the tubulin dimer many such tubulin dimers joined together to in a cylindrical fashion to form this micro tubulin. So, each green and blue together forms a single unit that you will in dimer consisting of alpha tubulin and a beta tubulin, it comes together to form this large macromolecular assembly which is called the microtubule.

The microtubule has various functions, it acts as the sort of railroad of the cell things get transported from one end of the cell to another by piggybacking on this microtubule network. It lends the cell some sort of structural rigidity. In fact, it is part of a class of objects which are called the cytoskeletal filaments, cytoskeletal filaments in that deform sort of the skeleton of the cell.

So, microtubule is one of these things. So, if we look at the micrograph of the cell this micro this microtubule network spans this sort of whole cell. An interesting thing about

microtubules is that these are not static objects they are not fixed they polymerize and depolymerise. So, here is my microtubule you could add tubulin subunits to the end and cause this polymer to grow which is this polymerization.

You could also remove tubulin subunits and you would get d polymerization. It happens in a very sort of signature way which is called when it goes from a polymerization to this it is called microtubule dynamic instability.

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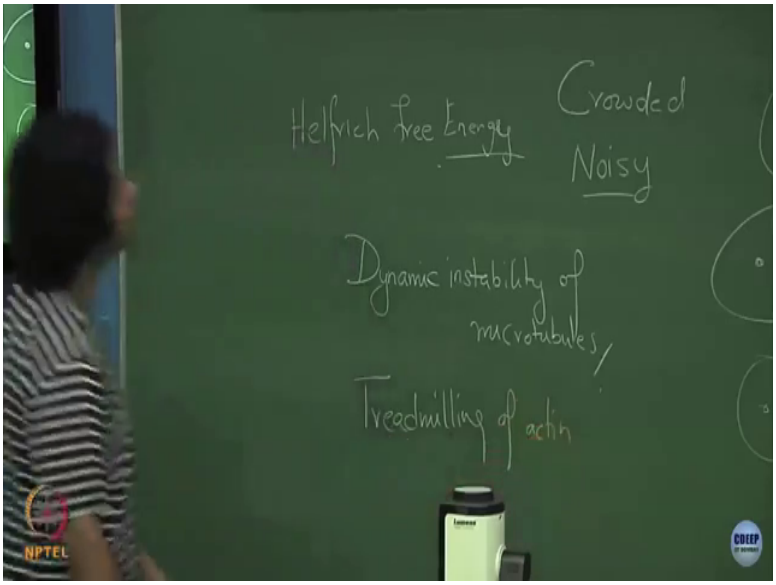

Dynamic it is called the dynamic instability of microtubules and again we will sort of look at this in more detail as the course goes on. So, I am just throwing various things out there this to get you familiar with the names and the terms as the course progresses we look at all of this in much greater detail and see how to model these various things.

So, when it switches from a polymerization state to d polymerization state it is called a catastrophe, when it switches back from d polymerization to a polymerization state, it is called the rescue event and again this is an energy driven event. So, when it attaches it has GTP. Once it attaches it hydrolyzes and the GTP becomes a GDP all right, so that is one micro macro molecular assembly.

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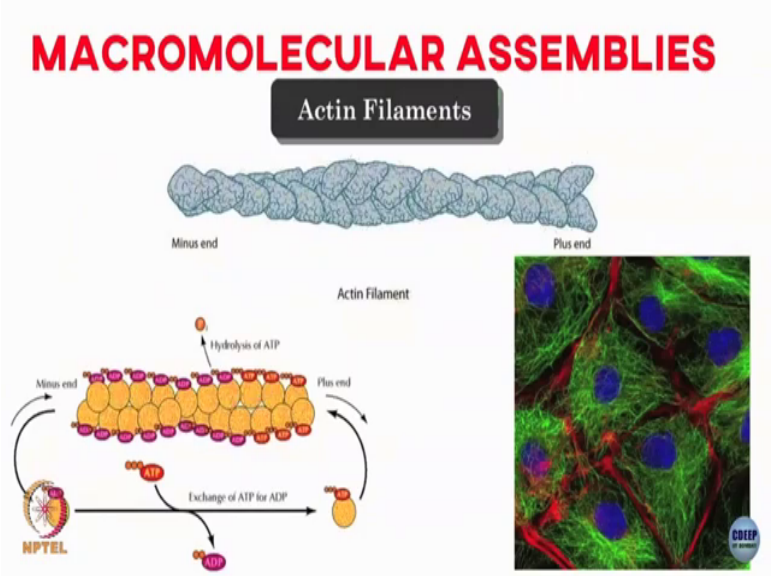

Another example is another class of cytoskeletal filaments which are called the actin filaments, and here again the subunit is something that is the actin protein, many such actin proteins joined together to form this actin filament. Again this is energy driven process, it is driven by this hydrolysis of ATP to ADP. Again these are dynamic in that for actins, you will get subunits being added to one end and being removed from the other end.

So, for actin the process is specifically called tread milling, it is called tread milling. So, these objects both microtubules and actin actually has a structural polarity, one end looks different from the other. And one end is called the plus end, one end is called the minus end these units get added to the plus end they get removed from the minus end.

So, again if you look at the micrograph of an animal cell, these green things these long green things are these microtubules, these red objects which are near the periphery of the cell they are the actin filaments. So, if you have this animal cell, you will have microtubules planning throughout this microtubule network. Then over here in the ends you will get your actins most. So, here you would form you would see this actins. So, again actin is another part of the cytoskeletal filaments.

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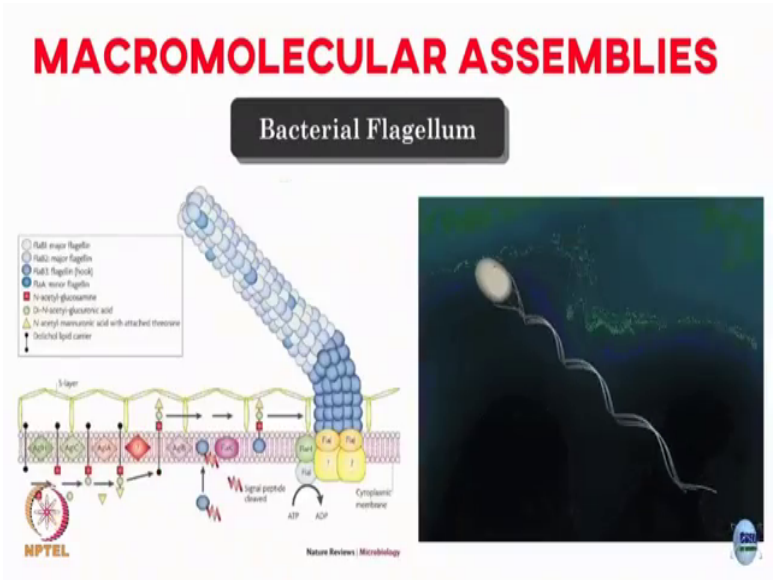

Another example of this macromolecular assembly is this bacterial flagellum which again is made up of many different proteins and so really fascinating thing which will again look at when we look at hydrodynamics. So, bacteria like the E-coli has many flagella this phospholipid drawing is actually a good drawing for E-coli flagella as well.

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So, here if this is my e-coli it has many flagella ok. When an E-coli wants to move in a particular direction all of these flagella has come together and they start to rotate in one direction in a coordinated fashion ok. So, it like a corkscrew it rotates and that causes the e-coli to move. When the E-coli does not want to move all of these flagella, so move apart from one another, so they are not bunched together anymore and the e-coli just drifts around. It is called two different phases of the e-coli motion run and tumble.

So, here is when the all these flagellas or sort of bunched together, they move in this corkscrew fashion and that propels the e-coli through the medium. Yes.

Student: When you say animal cell, what animal are we talking about?

E-coli.

Student: In the slide we had.

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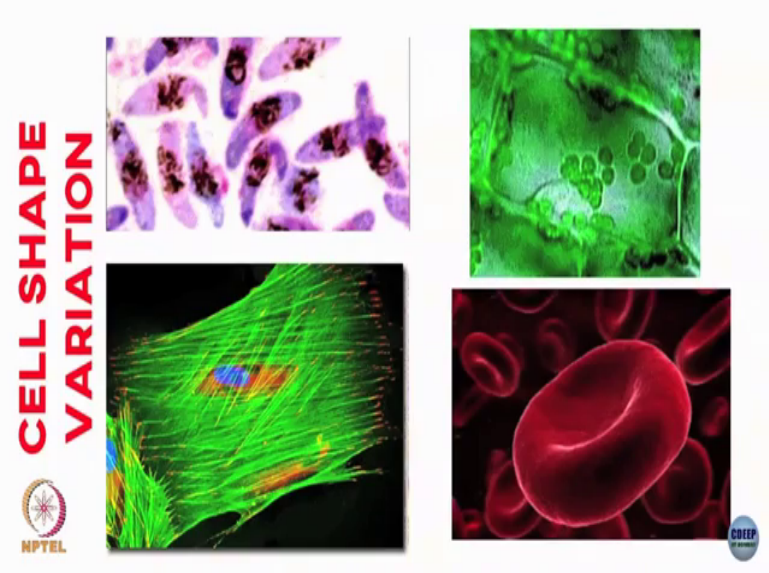

Long back I do not know which particular you are asking which particular cell type this is?

Student: Ah.

I have to look.

Student: Of which animal.

Of which animal, I have to look at this is a very generic picture in that sense in that it is if you look at the outer membrane it is sort of flexible it is not so plop the difference that I wanted to point out is that the plant cell is sort of roughly rigid. This looks like that rectangle whereas, an animal cell would look lovely like this as to exactly which cell this is a picture of I forgot I will look up and tell you ok.

But any generic animal cell will roughly tend to look like this sort of fluid floppy elastic membrane, a nucleus in the middle and then a lot of these microtubules and actin filaments all over the place. So, bacterial flagellum it is really the motor the bacterial flagella motor is really a marvel of nature, we will try to talk about this in a little more detail later on ok.

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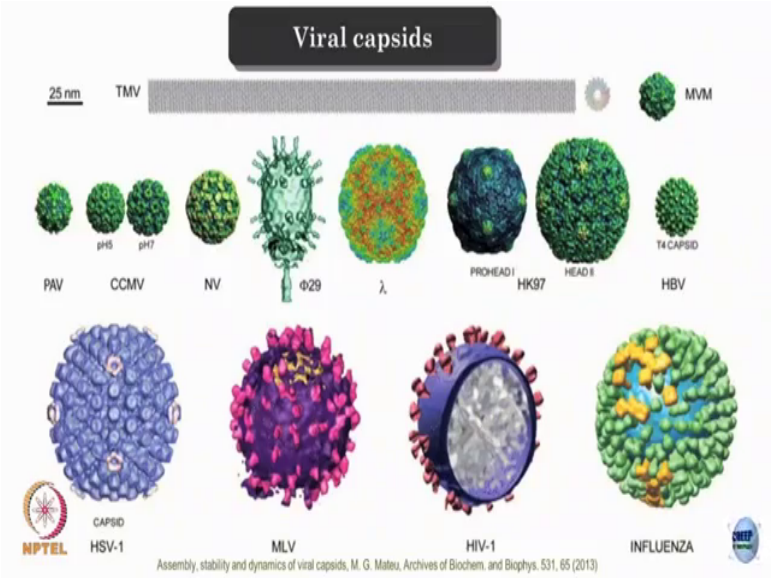

Again sticking with the sort of the theme of diversity here are a variety of viral capsids for from different viruses, here is the influenza virus, here is the HIV virus, here is the tobacco mosaic virus so on. So, you will see a lot of, so this is again another sort of macro molecular assembly, different proteins come together, they assemble the self-assemble to form this sort of capsules.

Some again some amount of modeling has been done in order to understand what sort of forces causes this assembly, hydrophobic forces are important, electrostatic interactions are very important. This individual protein subunits can be charged, they interact with DNA inside the viral capsids which are negatively charged, and this DNA can often cause a self aggregation of these viral capsules, at least for some viruses. Yes, somebody.

Student: (Refer Time: 26:49).

Good question, hold on to that, in a couple of slides I will come to timescales of the self assembly of capsules ok. I talk a little bit when I come to that these are different types of viruses.

Student: Nomenclature (Refer Time: 27:16).

The nomenclature of viruses mostly I think based on the disease, but I am not sure if there is some. So, I do not know want to answer, mostly based on disease for example, this virus causes influenza, this causes HIV and so on, but may not be true for example, I different people have named it differently I guess. This is a lambda phage, it is a random sort of sort of it ok.

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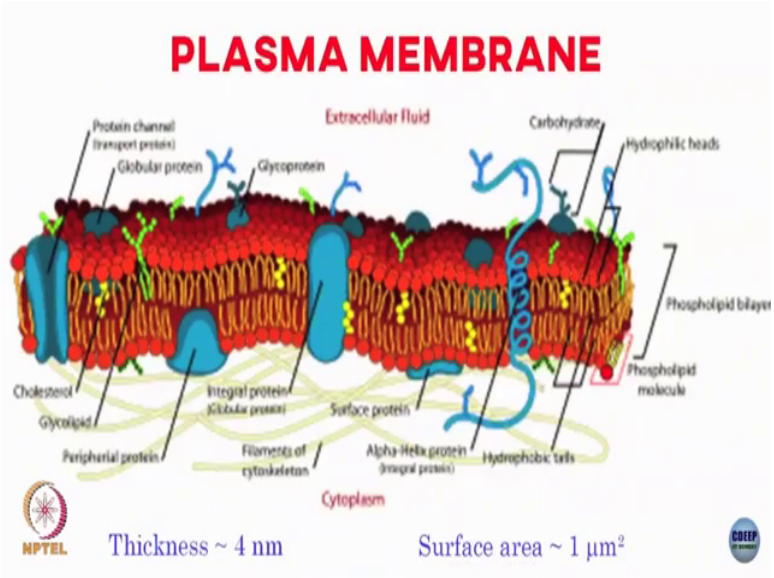

So, here is one more, so another assembly, so this is the plasma membrane. Again this picture is so bad. So, this is what I was trying to draw over there the phosphor is the phospholipids form a bilayer such that the hydrophilic parts are exposed to water, the hydrophobic parts are sort of protected inside. You get various sort of transmembrane proteins that are embedded into this plasma membrane.

You get other things like ion channels. So, this is one ion channel which allows things to move in and out of the plasma is a roughly around 4 nanometers thick and surface area of the order of microns.

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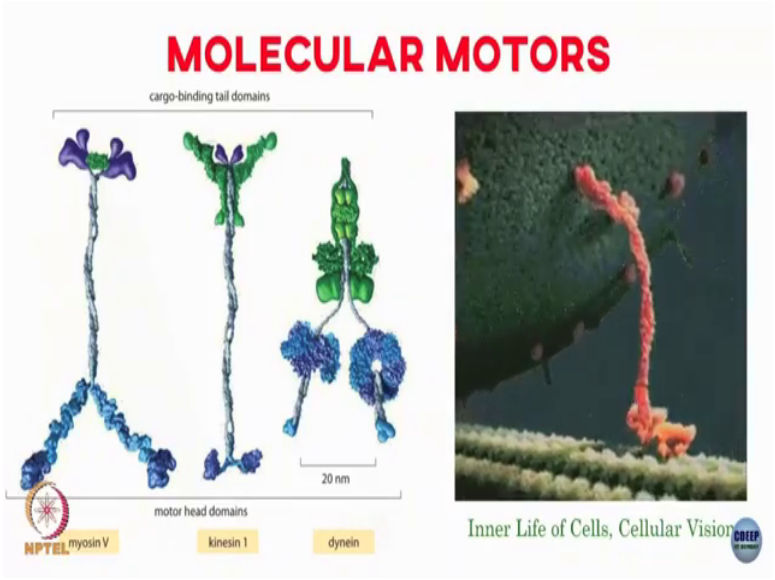

Finally, this is one more example. This is an example of a molecular motors. I am just showing sort of a random collection of things I find interesting. So, these are molecular motors which are proteins which walk on these microtubules, and the transport stuff from one end of the cell to another right. So, I said that these microtubules form like a railroad for the cell. And the rail way carriage as it were are powered by these molecular motors, they are powered by ATP consumption.

So, they consume ATP these subunits of the motors this head domains. So, this is these are three types of molecular motors – myosin, kinesin and dynein. I will just explain in a bit what the differences are, but this cartoon is for kinesin. So, you will see that these there are two head domains over here, these head domains attached to the microtubule and one by one they

unbind and they sort of walk. So, it is literally like walking on these tracks the other the tail domains bind to cargo, so that is what is being transported.

So, here it is a vesicle inside this vesicle might be different proteins that has been synthesized at one end of the cell maybe a protein was synthesized here, but its required for some function over here. How would it go from here to here it uses this railroad network, so it latches it goes inside a vesicle the vesicle is carried by these motor proteins which are walking on these railroad tracks.

Kinesins, so this is a kinesin motor, this is a dynein motor. Kinesins and dyneins walk on microtubules myosins walk on actin filaments. And again this is only one type there can be different subgroups of this, so there kinesin 1, kinesin 2 and so on.

Similarly, myosin 1, myosin 2, this is one particular type called myosin 5, but the basic structure is the same you will have a head domain which will bind to the filament whether it be microtubule or it, whether it be actin you will have a tail domain which will bind to the cargo and that will transport the cargo from one end to another.

These are intrinsically driven processes these are non-equilibrium processes driven by the consumption of energy you need ATP or GTP or some sort of an energy currency in order to transport these in order for these motor proteins to function properly.

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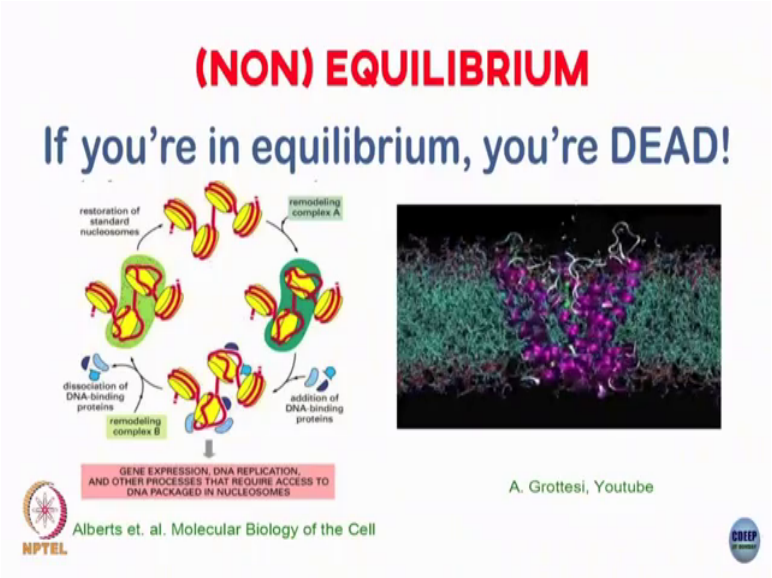

In fact, this sort of concept of non equilibrium is something that we will look at in a little bit of detail towards post mid-sem, basically most processes inside the cells are non-equilibrium processes at some levels they are driven by energy. In fact, they like this court that if you if as a biological system, you are in equilibrium that basically means that you are dead.

Anything in order to be living you need to be consuming energy you take in food that gets converted to all of this ATP, GTP which the cell uses to perform all of these tasks. Does that mean that whatever equilibrium calculations that we will do pre mid-sem are not correct, of course, not you just have to be aware of the limitations of that, and that is something that we do not in biology, but everywhere for example, if I were to say that what is the temperature of this room I do not know maybe it is 24, 25 degrees right, but it is not really the it depends on what time scale you are talking about.

If you are saying what is the temperature of this room over a scale of 24 hours, the temperature is not constant right its changing the room is the non-equilibrium system its exchanging energy of the surroundings and so on, and it is changing temperature. But if you are looking at a period of time with its non-equilibrium properties are not important, you can define a temperature.

Similarly, you can do equilibrium calculations depending on what the timescales and the spatial scales are and that is what we will spend our time in the pre mid-sem part of the course. We will look at equilibrium keeping in mind in the background. Yes.

Student: (Refer Time: 32:44) because we keep consuming and the species in a chemical biochemical reaction networks.

Hm.

Student: are always changing I mean like concentrations are always changing, so is that why (Refer Time: 33:04) go to equilibrium.

Sorry, I did not understand.

Student: So, we a cell has biochemical reaction.

Yes.

Student: Inside right.

Yes.

Student: Is it because the cell is always getting species inside?

Hm.

Student: And that is why it is not reaching equilibrium.

It is a non-equilibrium system because in order to maintain, the proper functioning of the cell which is all of this the chemical reactions, reproductions or DNA duplication and so on and so forth protein production all of this required energy. The moment you stop producing stop providing energy to a cell the cell will stop performing all of these things, it will stop the biochemical reactions, it would stop protein production and so on and so forth, the cell would die ok.

So, what basically what is equilibrium you take a system, you leave it to itself, it reaches a nice happy state on its own right. If you took a cell and you did that the cell would die, you would have to constantly supply an input of energy in order for the cell to perform its functions. So, in that sense the cell is intrinsically a non-equilibrium system ok.

Does that mean that whenever we talk about any chemical reaction that is going on we will never use equilibrium approximations? No, we will use keeping in mind that these are a small subset that we are looking at the whole network if you were to look at the whole systems biology of all of these reactions going on overall it is a non-equilibrium system, some small part may be some small constituent part may be in may be in equilibrium for a small duration of time for the duration of time of that reaction ok.

So, that is what. So, we will talk about equilibrium and we will try to clarify when the equilibrium approximation is appropriate and when it is not, and when it does not will try to see what sort of, we will try to see to a small extent what sort of things we can do.