**Physics of Biological Systems**
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**Lecture – 23**
**Energy and equilibrium**

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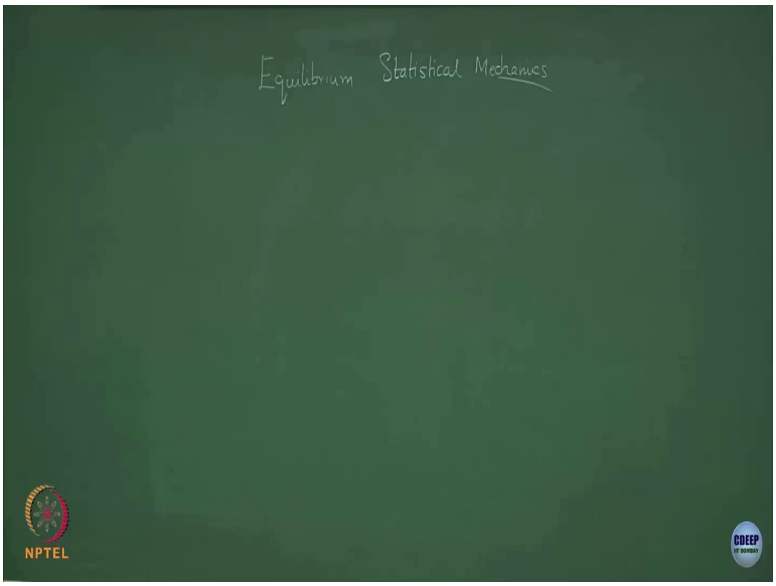


So, before I will do some simple calculations, but before I do; we will just sort of emphasize once more, how and when equilibrium considerations might be appropriate. We know overall all biological systems are non-equilibrium, but in certain cases; one might get away with an equilibrium approximation. So, we will sort of discuss; how this a certain whether an equilibrium approximation is good or not.

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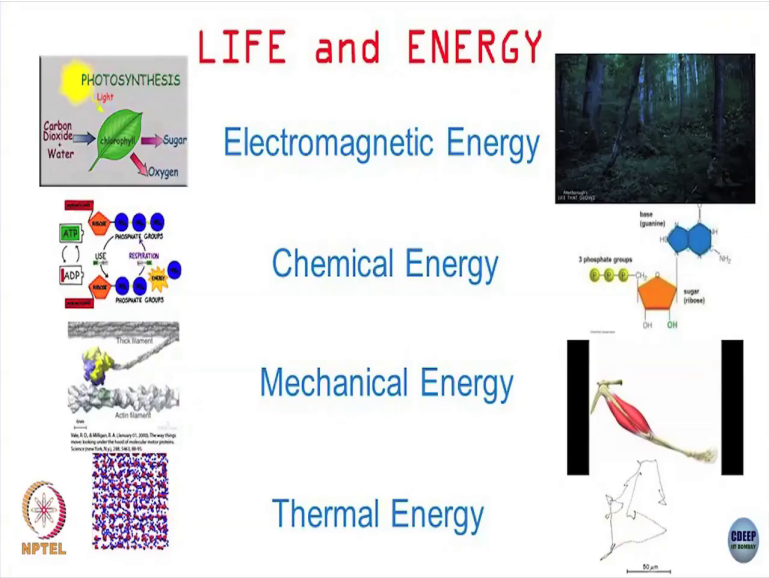


Before we do, so let us just start. So, energy in inside cells comes in various forms of course, what we have been discussing is in some sense the manifestation of thermal energy right diffusion, viscosity and so on. So, Brownian motion and so on. All of these are manifestations of thermal energy. But, there are other forms of energy that are relevant to a living system; for example, chemical energy right ATP is a form of chemical energy.

So, you store chemical energy in ATP, use the ATP hydrolysis to get that useful energy out and do some work. What other form of energy would be relevant? Electrical energy, let me just generalize it and say electromagnetic. Where would that be applicable?

Student: (Refer Time: 01:48).

Student: Nerves.

Nerves that is good. What else?

Student: (Refer Time: 01:58).

Pressure. So, let me say mechanical energy and then one can look at different manifestations of these. For example, is electromagnetic energy one of the most basic manifestations where electromagnetic energy is important; is for example, photosynthesis right. You take in sunlight you convert that into useful energy which is basically the basis of all energy for life forms on earth, plants do that and then different things eat plants and we eat different things and so on.

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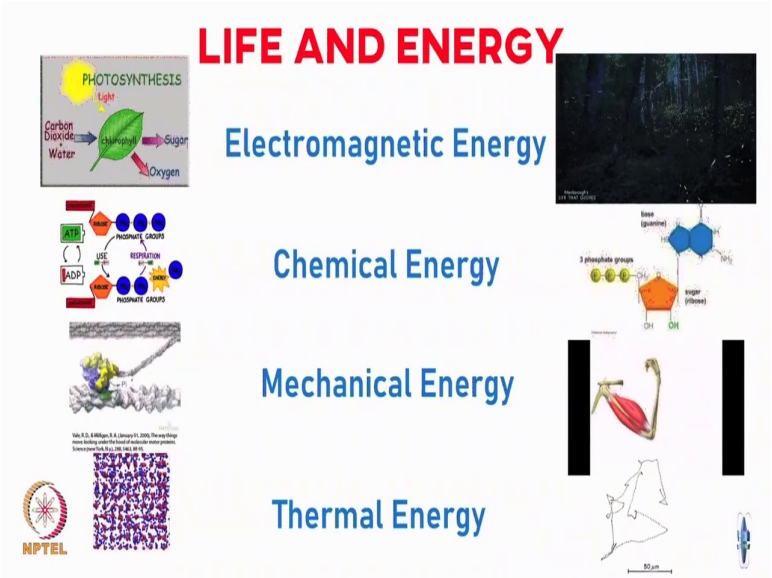

Also for example: these fireflies which are glowing in the dark. These also use stored chemical they converge stored chemical energy into this light energy and they blink and they give up light. So, this is sort of the reverse of this where you take in light and you store it in the form of chemical energy these fireflies are out bioluminescent animals in the sea and so on. You convert chemical energy into a palm fly. Chemical energy; we stopped off for this ATP GTP and so on.

You go from an ATP to an ATP and in the process you get energy. Mechanical energy for example, you can think of these muscles flexing in your arm or even molecular motors which are pulling on different proteins. So, in this case; this is a myosin motor which is acting walking on actin filament and pulling an actin filament and then of course, there is thermal energy of diffusion and random walks and so on; so, that is ok. So, that is to do with energy before I actually get into equilibrium and so on.

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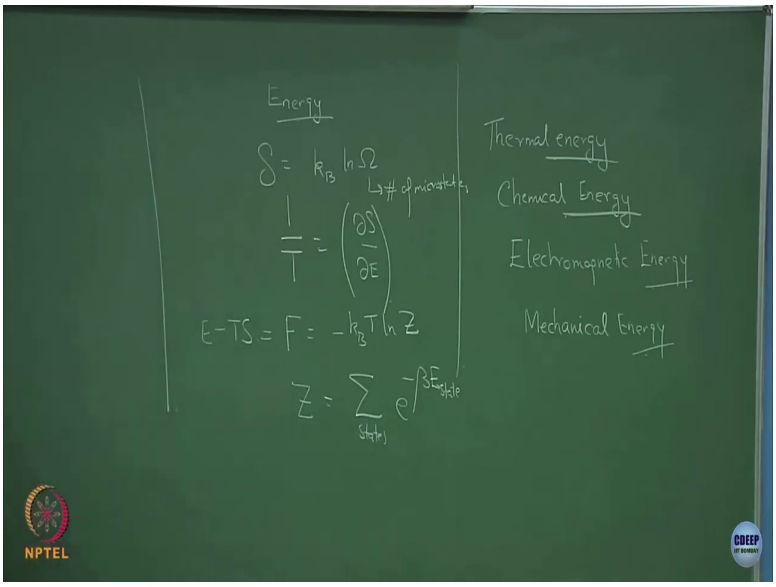


Let me just, so energy we are at least all familiar with. What is entropy? What is the microkernel, what is the statistical definition, statistical mechanical definition of entropy from a micro canonical ensemble? It is kB times log of omega right. Well, what is omega? Number of microstates that correspond to a given macro state; so, this is the number of microstates.

What is temperature? Yes, what? Nothing I am going to forget what is temperature is 1 by T is del S by del E right. What is the Helmholtz free energy? Minus kBT log Z good. What is the partition function? Z, it is the sum over all states, e to the power minus beta times E of a state right. Then this F is minus kB T log Z or you can also write this F is E minus TS good. It is just to keep in mind.

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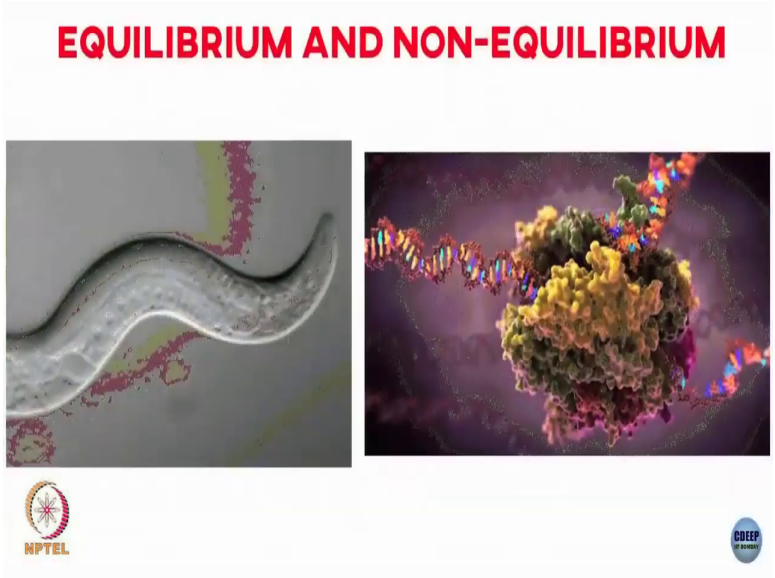


So, here are for example, two processes which are definitely non-equilibrium here is a worm which is crawling across the surface. Here is an RNA polymerase which is reading a DNA molecule and producing the corresponding MRA both of these are reliant on energy. RNA polymerase uses energy to slide along the DNA, the worm of course, uses energy to crawl along the surface.

But, depending on the question that you are asking in the non equilibrium nature of this overall process may not for example, the important. For example: if we ask that well given certain concentration of RNA polymerase molecules in the cell. What is the probability that the RNA polymerase will be bound on to the DNA ok. So, here is my DNA, here is my double stranded DNA. It might have different binding sites for RNA polymerase right; which are upstream of the transcription genes gene sites.

So, now any polymerase can bind come and bind here and then it can read this read this DNA sequence and produce an MRNA the sliding is along the DNA. So, non is active or a driven process which requires anything, but for example, if I am not interested in this whole problem.

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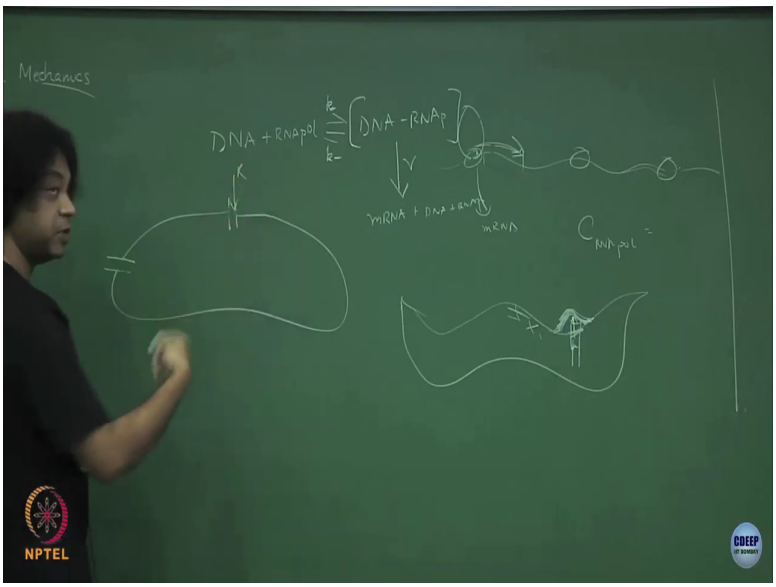


But, if I just want to ask that what is the probability, that an RNA polymerase molecule is bound to this DNA backbone; given a certain number of for example, binding sites given a certain concentration of RNA, RNA polymerase and so on. This question as we will see; that is one of the questions we will try to answer. You can sort of deal with within an equilibrium approximation ok. The probability that a certain site is occupied by an RNA polymerase is can be obtained or can be predicted fairly well within equilibrium approximations.

Similarly for example, if you are looking at this sort of deformation of the cell the cell shape underneath the structure of this form you have whatever you have this cytoskeleton which is

composed of actins and microtubules and so on. These exert forces on this cell wall on the membrane and they can lead to deformation of this deformation of this of this membrane. The forces that are generated by the cytoskeleton are explicitly an active process. These are driven by motors which walk on these filaments and generate forces.

These, but if you are looking at the structure of this the shape of this you might say that well; if the timescales if this feels a force, it responds to this force and assume some shape. If the time scale of that process happening is very different from the time scale of this motion of this organism of the cell as a whole then we can consider this as in sort of equilibrium problem. I can say that when I apply a force what would be the same shape that this membrane is used. So, again if you look at some sub part of the problem where the time scales are different from the time scales where these non-equilibrium factors become important it is often reasonable to use an equilibrium approximation to show this you can explicitly consider.

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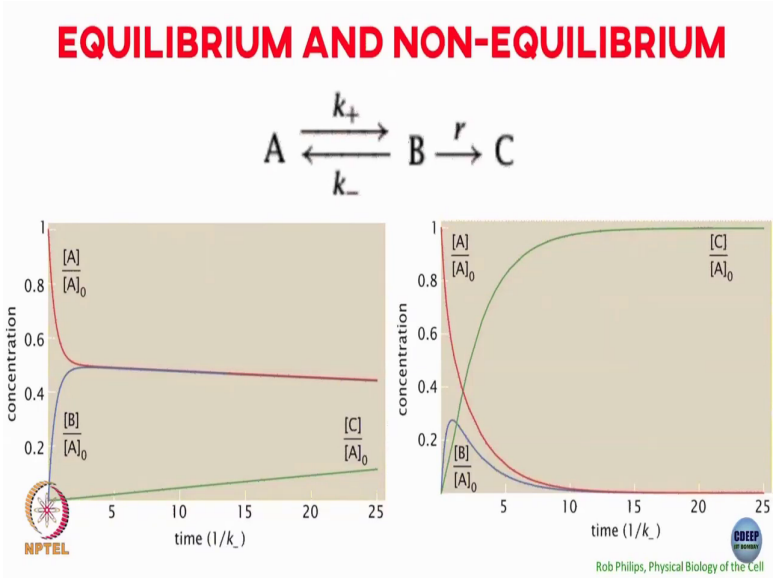

So, let us start give an example of a chemical reaction. So, for example: if I take a chemical reaction like this right. I have A going to B with some rate k plus, B converts back to A with some rate k minus and it also goes to C with some data ok. There is no reverse thing over here. So, for example, this could stand for consider this as this RNA polymerase thing only, you could have DNA plus RNA pol going to this complex formation where this DNA is bound to the RNA polymerase is right.

This is inter-convertible the RNA polymerase binds and unwinds right. So, it is a reversible process, it goes forward and backwards with some rates. But, this one will also give you with some rate r let us say the corresponding mRNA plus the DNA plus the RNA with that right. So, it is something this is of course, it is not to say that this is just represents this process this is just an example, you could consider many other reactions which is something like this.

Now, if they are these rates the time scales that are associated with these rates k plus k minus and r are very different. For example, if this k plus k minus this A to B inter-conversion is a very fast process whereas, this b to c which is given by this rate r is a very slow process. If you just consider something like that and then if you try to solve that if you try to see; how these molecules will change. The concentrations of these molecules will change with time that will look something like this.

Again, if this is a given we will solve it along the way as we go along. So, for example, this red is the concentration of A this B is the this blue is the concentration of b and this C is the concentration of this green is the concentration of C right. So, in the limit where these inter conversion of A and B are very fast processes right. k plus and k minus are much faster than this rate r. A and B will come to an equilibrium amongst themselves. This does not mean that a becomes constant with time as you can see the concentration of A changes with time B also changes with time.

And therefore, C also changes with thing that happens over a much longer time. But over this very very quickly, over this very short timescale A and B have come to an equilibrium amongst themselves; which means that the ratios of a to the ratio of b assumes a constant val

that constant then changes with time, but A to the ratio of A to B stays constant that is a reflection of the fact that; you have very two very separate timescales in the process. One time scale which is given by 1 by k inverse k inverse k plus or k minus which is this time scale. Let us say 2 3 seconds.

The other is the time scale which is given by r inverse, which is a much longer time scale say 50 it is 100 seconds and so on. So, if you have well separated time scales like this, then and if you are interested only in a sub part of this whole process if you are only interested in this a converting to B sort of be processed and not this whole B going to same thing then you might be with considering this A A inter converting to B as an equilibrium process. And then using equilibrium (Refer Time: 12:34) to calculate statistical quantities associated with that.

This depends; of course, on whether these time scales are well separated if this k plus k minus was not a much faster process which is given in this right panel. Then of course, it does not come to an equilibrium. You will see that; A you know varies on its own B which is the blue curve varies on its own c varies on its own and so on ok; so, the same. So, depending on whether you can separate your time scales for these sub processes, you can consider these sub processes as part as an equilibrium under an equilibrium approximation ok

So, that is the basic idea. It always or mostly has to do with separation of time scales you identify time scales that are widely separated and one is much smaller than the other then you might be justified in using the equilibrium approximations.

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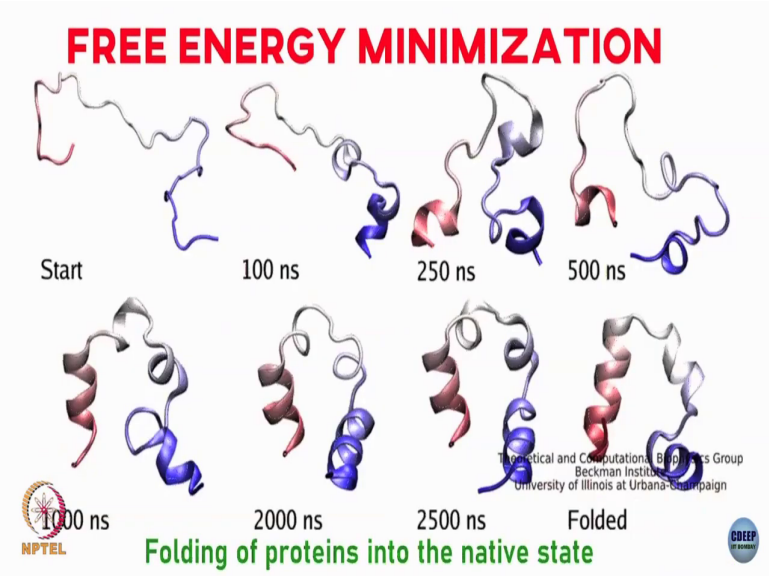


What can you do with such things one of the classic problems equilibrium problems that one attempts is finding the folded structure of proteins. So, you start off with a chain of proteins which is some amino acid sequence and then the protein manages to fold into this final native structure or the functional structure very quickly; so, some 2500 or 3000 nanoseconds.

This is still of course, an open problem it is an extremely hard problem as to how given a sequence you can end up at the structure, but we will again look at how to at least start thinking about these problems.

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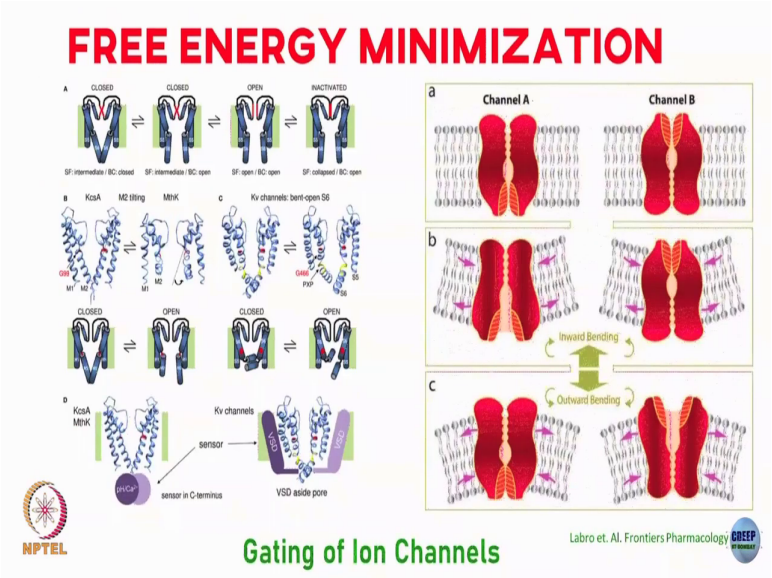


Similarly, another example where you might consider free energy minimization is to do with gating of ion channels. So, ion channels are these channels on the membrane of a cell which lets in ion. So, here is my cell here is an ion channel here is an ion channel and so on. And you can have different ions for example, potassium, sodium which pass through these channels. These ion channels can be open or closed; if they are closed they do not allow an exchange of their open the law line exchange.

For example, here is one mechanism which you might tell which is one of, so self control the opening and closing of these ion channels in different ways. So, for example, this on the right hand panel is a way where you control it using mechanical tension in the membrane right. So, here is an ion channel here is the membrane in which it is embedded this is the lipid bilayers

membrane; depending on what forces or tensions you apply on the membrane you will result in a different free energy.

So, for example, here is some membrane; which has an ion channel which is closed, let us say nothing can get in. Due to some reason you apply some you apply some tension on this and maybe it switches to by a free energy minimization under the effect of this tension may be the shape changes to something like this; where the ion channel becomes like so. So the structure changes from here to here and it goes from a closed to an open state.

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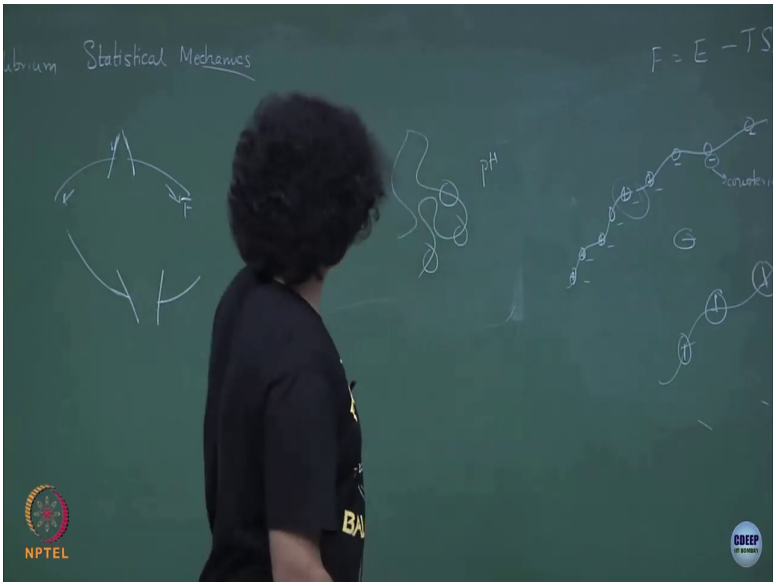


So, again you can think of this in terms of a free energy minimization in the presence of some forces, or you could have voltage-gated ion channels where you change the voltage across on the two sides of the membrane. And that again causes the ion channel to go from a closed state to an open state.

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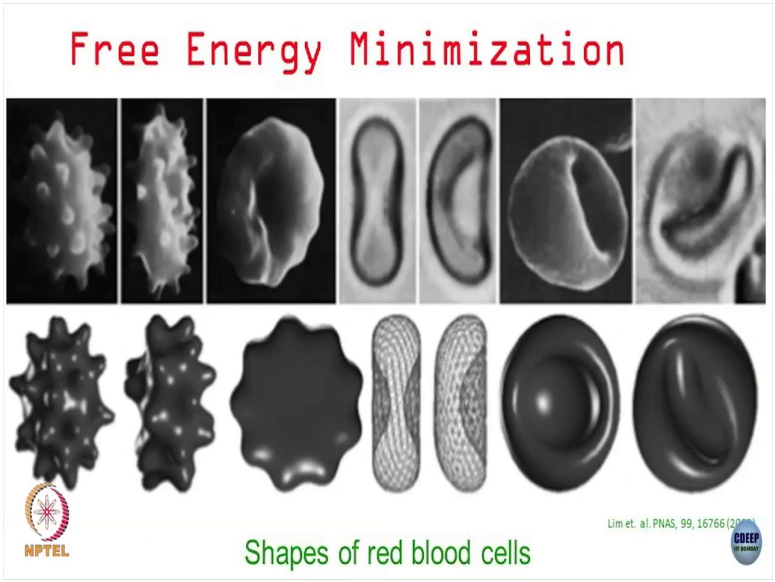


Then of course, we have discussed this earlier the shapes of red blood cells is a classic problem where you can get the canonically observed shapes are close to the canonically observed shapes through a free energy minimization. You write down the Hamiltonian, which takes into account the physical forces that are involved bending surface tension and so on. You minimize that free energy you minimize that free energy for different values of this parameters which might be the bending energy strength the surface tension strength and so on.

For different strengths of these parameters values; you get different shapes is the minimum of the free energy and you can show that the shapes that in these different shapes that you obtain closely correlate with the shapes of red blood cells that are seen in vivo.

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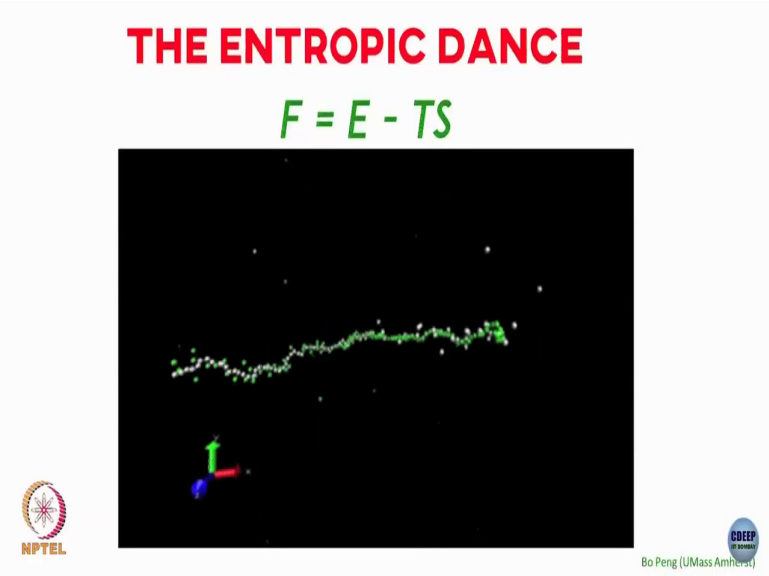


So, underlying all of this is the competition between energy and entropy. So, this free energy of encompasses two terms the energy term E and the entropy term minus TS. So, for example, this is a simulation of a poly electrolyte; which is so let me just describe yes. Can you see the protein folding happening in real time? I do not think so, right. You can get X-ray crystallographic structures; which are the folded structures and then you can reconstruct, at least you can get that for small domains and so on.

You can reconstruct the 3 D folded shape I do not think you can see the have the sequence folding into the shape in real time. You can see that; well, you cannot see, but to a certain extent maybe in molecular dynamic simulations; if you trust your force fields that they are the accurate force fields up to a few nanoseconds for not too large proteins you can see how it starts folding, but there is no guarantee that that is how the cell is doing. So, for example, here let us see I take a polymer; it could be any charged polymer DNA or even proteins so on. So,

which has some backbone. So, these are the monomers of polymer and these are these have some charge loops sitting on them right negative charge is sitting on them.

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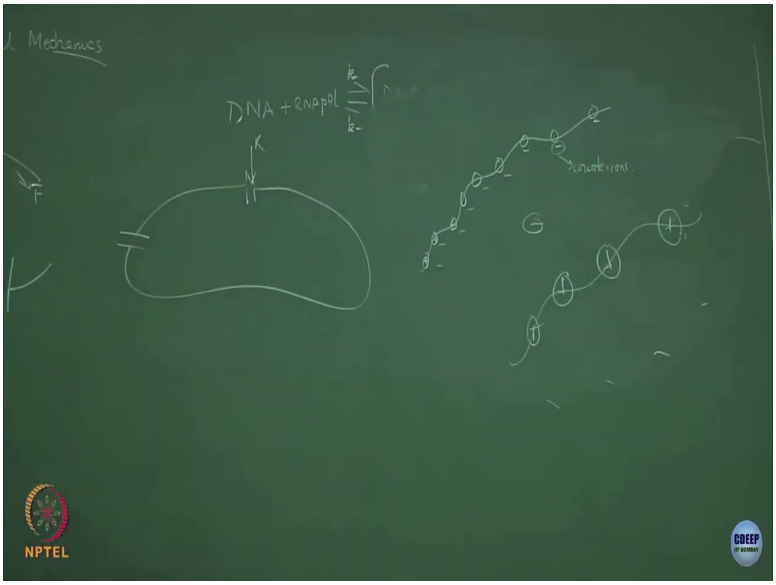


If these, so these are called the counter ions. So, these are my electrons, these are my counter ions. One could ask that, what is the effective charge of this polymer. So, for example, if with each monomer if each monomer is plus 1, it is associated with the counter on it is minus one over all this polymer is neutral right. But, this counter ions can dissociate and go in solution right, when you put it in solution this counter ions tend associate with leaving a polymer which is say very strongly positively charged. And, the counter ions are all floating around freely inside (Refer Time: 19:11) right. How much of these counter ions will?

So, how much of this counter ions will dissociate; it is sort of a competition between energy and entropy. For example, if the counter ions sit here then it gains some energy because, there

is an interaction between this plus charge on the backbone and the minus charge of the counter all right. So, it lowers is its energy by sitting here on the backbone which is good it wants to lower its energy, but on the other hand if it goes into solution it can increase its entropy right.

Because, here it is fixed over here; it does not, it cannot explore around if it goes in solution it has this whole solution space to explore around. So, it gives up some energetic it gives up some favorable energetic cost which by associating with this plus, but on the other hand it gains an entropic cost. So, which or how much of these counter ions will go into solution, then is determined by this free energy; which is E minus TS right it is a competition between the energy and the entropy.

So, for example, in the semi richness; these are two polymers were carrying opposite charges and initially the counter ions were very close to the polymers what will happen is that this polymer will come in. And, it will display it has a stronger binding affinity to these to this first polymer and eventually it will displace all the counter ions which will start going into solution and these two polymers will form a complex.

But this is also true for example, in the case of proteins. So, for example: if you have a protein right. The amino acids the amino acid groups on the protein can be charged or not; depending on what is the ph of the sound. So, let us say I have some folded protein which have some amino acid residues. Depending on the pH of the solution; you can change the effective charge on these amino acids right.

So, depending on the pH, you can change the effective charge on these amino acids. Once you change the charge; you might also change the structure right, because that will change the forces that are felt by different parts of the protein and therefore, you get a different free energy minimum right. So, that is why often you will see that there is not a unique native state for proteins; if you change the ph of this of the environment that this protein is living in. It can go from one folded confirmation to another. So, these would be yes.

Student: (Refer Time: 21:32).