Physics of Biological Systems
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Lecture – 35
Deriving the full force extension curve

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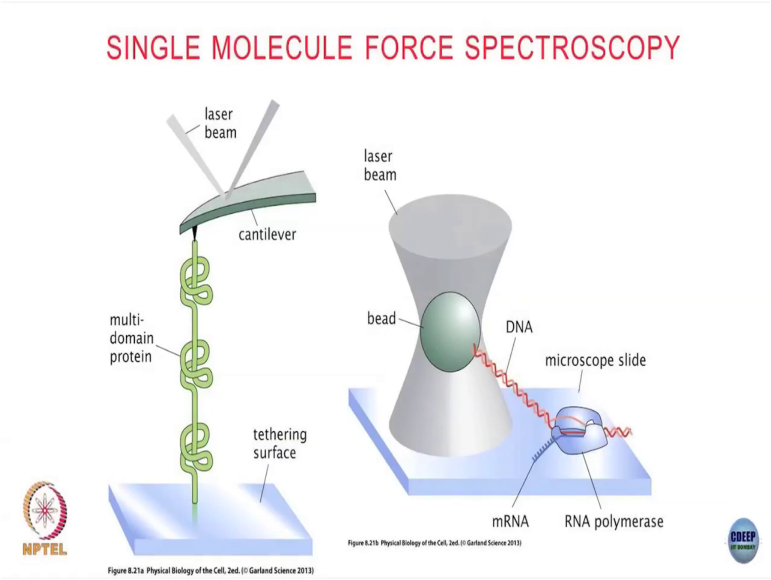

SINGLE MOLECULE FORCE SPECTROSCOPY

Figure 8.21a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Figure 8.21b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

So, these are single molecule experiments. Here for example, is a protein that I have fixed one end to a tethering surface. And then using an AFM tip, you pull the other end of the protein right. And you can plot a similar curve like this that as a function of how much force you apply what is the extension of the polymer right or you can use something like an optical trap to pull again to again to apply exert forces. What you get is something that looks like this at least in the initial part, but then of course, it deviates because this one, we argued only for very small extensions.

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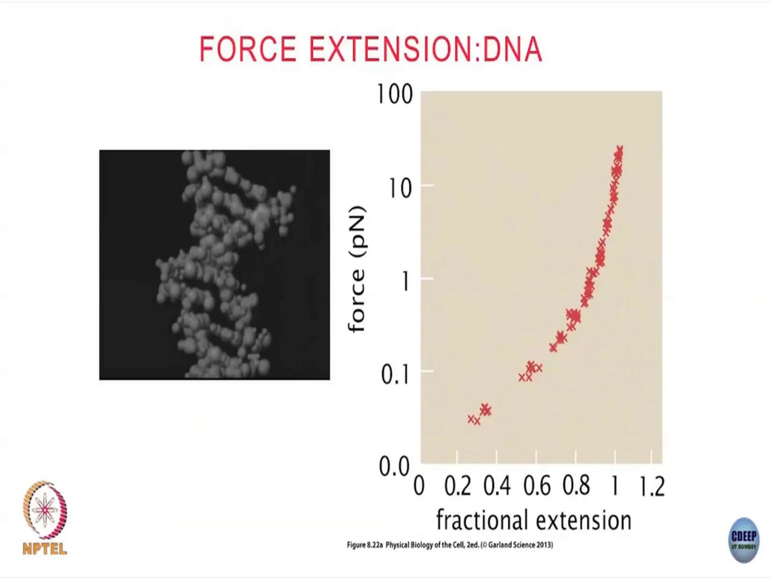

FORCE EXTENSION:DNA

Figure 8.22a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

So, if you look at DNA for example, so here is a DNA let us say equalate using one of these AFM tips or an optical trap or some other setup what you see is a force versus extension curve like this. Initially for very small extensions it sort of grows linearly but then, as we argued that after all you cannot stretch the polymer indefinitely this extension has to be less than equal to the total contour length of the polymer.

So, therefore, as you pull it more and more as you apply more and more force the curve will become more and more non-linear. And you cannot go beyond this one on this line right; because that is the maximum possible extension of this; maximum possible extension of this curve. Of course, as you keep pulling at some point the polymer will break, but let us say we are not getting into that region, but this is how what the curve will look like.

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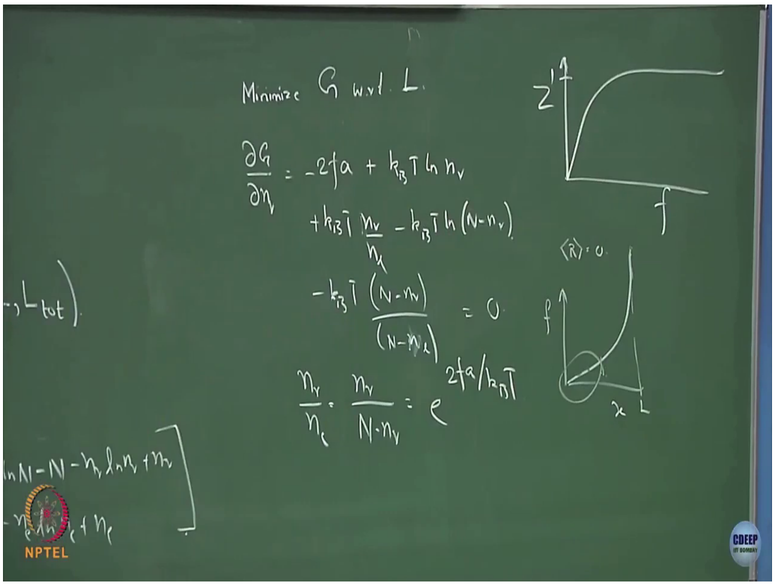

Minimize G w.r.t. L.

$$\frac{\partial G}{\partial n_v} = -2fa + k_BT \ln n_v + k_BT \frac{n_v}{n_v} - k_BT \ln (N-n_v) - k_BT \frac{(N-n_v)}{(N-n_v)} = 0$$

$$\frac{n_v}{n_c} = \frac{n_v}{N-n_v} = e^{2fa/k_BT}$$

$L_{tot})$

$\ln N - N - n_v \ln n_v + n_v$

$- n_c \ln n_c + n_c]$

So, force versus extension will look something like this right; this being the maximum possible extension. What this does is and if you do it for so, this response of this polymer depends on the particular polymer that you are pulling. For example, if you use two different DNA sequences and you pull them you will get a slightly different force characteristics ok.

So, this risk this forced extension curve is sort of what is like the fingerprint of the polymer that you are pulling. So, it is a characteristic of the particular polymer that you are pulling. And therefore, it acts like some sort of fingerprint ok; if you pull a different sequence of DNA would get a slightly different curve and so on.

Now this part at least we understand that this part is the linear part which we argue would last class. But we can try and see if we can derive this force extension curve in the full limit, without using this small extension approximation ok.

So, that is what I will try to do first and we will see whether we recover a plot like this ok. So, let us see so first I will do it for a 1 dimensional chain and again then I generalize to a 3D polymer. So, first I will do it for a one week only. Remember that the free energy is like E minus J dot x minus TS right.

I am using the Gibbs free energy in this case because I will be working in ensembles where this J remember is what is a generalized force x is a generalized displacement right.

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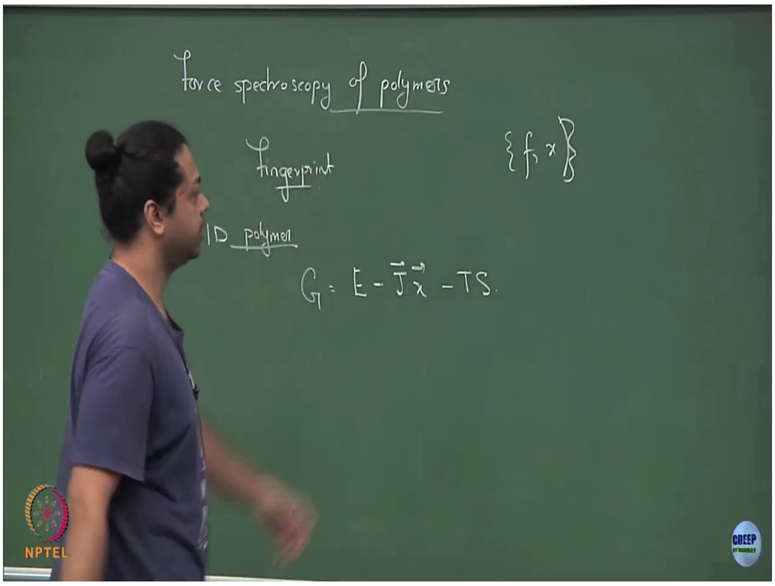

So, like pressure and volume and so on. So, in this case the core the pair that I am interested in is the force versus the extension I have written x for the extensions. So, I will stick to that the force and the extension ok. So, we apply a constant force and the extension sort of responds to this force that I am applying. Of course, you can see that as I pull this polymer more and more, my entropy sort of decreases because the polymer has very little the configurational entropy that is available to it.

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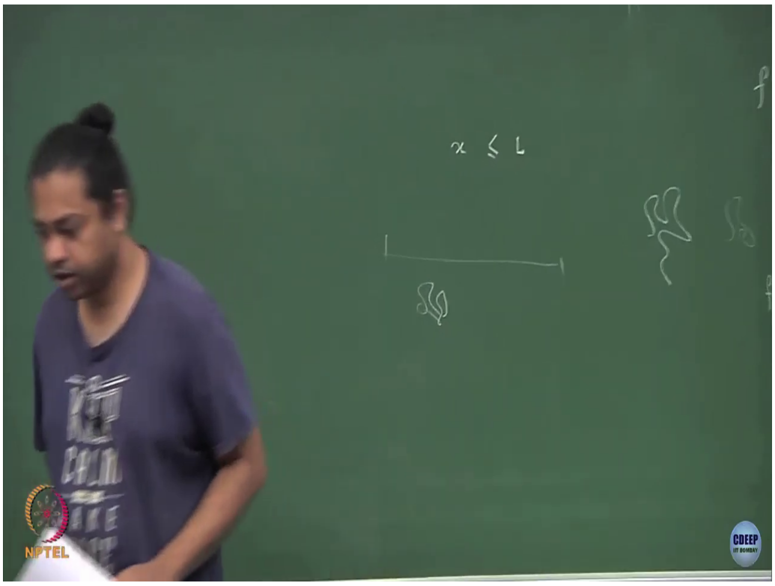

If I were to pull it completely ; it has nothing to do except be in a straight line if it was if I did not pull it at all it has a lot of entropy among the number of available microstates and you will. So, let me get rid of J and x. So, let me just write f and L ok.

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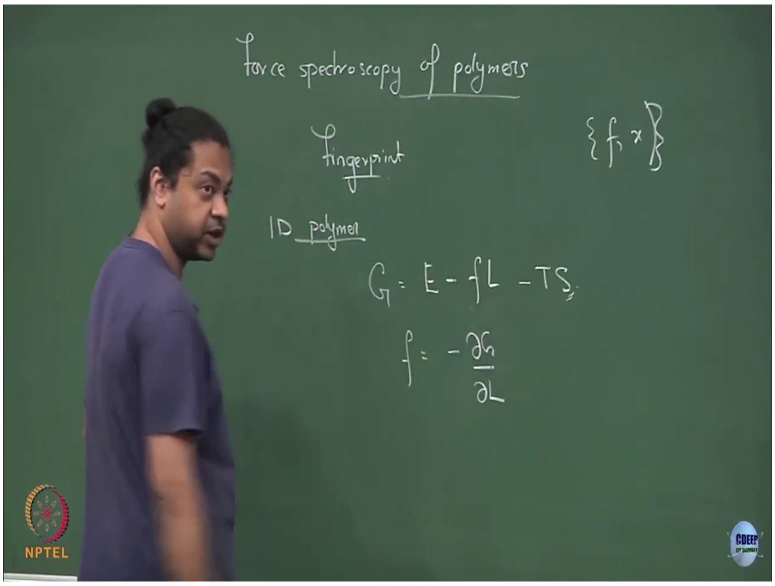

This is a those are the appropriate things in this case. And the force as you again know is given by minus del G del L right, just like pressure would have been del G del B exactly like that the force is minus del G del L. Remember pressure has an ad hoc extra negative sign because of how the original convention was defined. Every other generalized force and generalized displacement will come with the negative sign so, that is what we will try to do.

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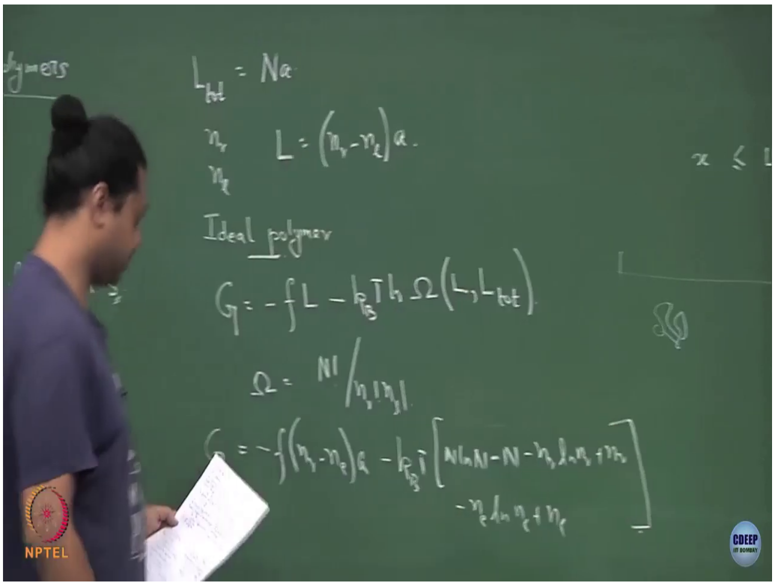

$L_{tot} = Na$

n_r $\quad L = (n_r - n_l)a.$

n_l

Ideal polymer

$$G = -fL - k_BT\ln\Omega(L, L_{tot}).$$

$$\Omega = N! / n_r! n_l!$$

$$G = -f(n_r - n_l)a - k_BT\left[N\ln N - N - n_r\ln n_r + n_r - n_l\ln n_l + n_l\right]$$

So, let me formalize. So, let us say I have a polymer a 1 dimensional polymer with the total contour length of N times a right N is the number of segments that I have a is my contour length or the length the size of a monomer and. So, therefore, that is mine l total this is a 1 d polymer.

So, I just have two possible options I can go take a step to the right or to the left and those are given by n r and nl. And therefore, the length of the polymer the end to end distance so, the end to end distance is nothing but n r minus n l times a right. How much excess of one over the other that you have?

And remember, I will what I will be doing is this sort of random works are the polymers. It is an ideal polymer which means that there are no interactions between the monomer segments; which means that I do not really have any e right. In reality of course, there is some bond energy and so on, but at least for this ideal polymer I assume that there are no interactions between the different bonds ok.

So, then I can write down my Gibbs free energy, my Gibbs free energy is minus f times L and minus ts. So, minus k B T log of the number of microstates ok, the microstates for this given extension L given that the total possible extension the maximum possible extension is L total ok.

Now, I know what this omega is right, we have done this before. So, my omega is simply how to distribute these N monomers such that nr in or on the right and nlr on the left. So, that is my N factorial by n r factorial nl factorial. So, we have basically everything I have my L in terms of nr and nl I have my omega in terms of my nr and nl. So, I can just put everything over here and calculate what is therefore the Gibbs free energy. So, let me just write that down. So, the Gibbs free energy is going to be minus f times l; so, nr minus nl a. And then from this part, we will get minus k B T N log n minus N minus nr log n r plus n r minus nl log nl plus.

Right now, what I need to do to find out the force is basically two minima to find out this derivative of this free energy with respect to the length. Or equivalently what I will do is I will take a derivative with respect to nr; because these are basically the same things. So, I want to minimize my free energy. So, I want to minimize this free energy, this free energy with respect to my length of the polymer L.

So, let me these two del g by del n r and that is minus 2. So, this I will just write down you just check that the mathematics is correct. It is a simple derivative plus k B T log of nr plus k B T nr by nl minus k B T log N minus nr ; minus k B T N minus nr by N minus n l. Actually I think this is yeah and if I now set this equal to 0, what I will get is the relation. So, what I will get if I solve for n r is that nr by n l is equal to so, this is basically nothing, but n r by N minus nr nl is nothing but N minus n r. If you do the maths correctly, we should come out to be 2 f a by k B T which means that, the extension or let me derive; let me define the relative extension rather because that was what it was plotted over.

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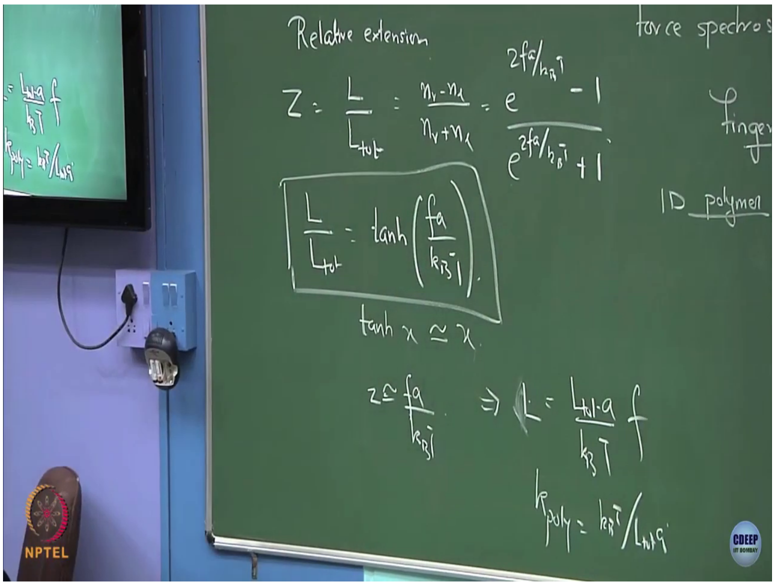

There the relative extension is let me say Z is nothing but L by L total ok. So, this can vary between 0 1 1 and this is nothing but l is nr minus nl l is nr minus n l and l total is nr plus nl the as cancel each other. And then if I substitute that solution, I got for nr and nl what I will get over here is e to the power of 2 f a by k B T minus 1 by e to the power of 2 fa by k B T plus 1.

And this is a simple function what function is this? There is nothing but the tan hyperbolic right. So, this is nothing but tan hyperbolic fa by k B T ok. What is the tan hype what is tan hyperbolic x for small x anyone remembers? For small x tan hyperbolic x is just x; which means that for small forces, you will just get that the relative extension z is simply nothing but f a by k B T right, which again tells you that the extension is proportional to the force.

So, we recover back the result that we got in the last or last two last class that if I were to write L, then there is nothing, but L max or L total. Whatever I am writing l total times a by k

B T into f right. And then that give me my spring constant is k B T by L total into a which is what we had last class. So, that gave me my spring constant of the polymer was k B T by L total Q.

So, this so that is a consistency check that whatever we have done in the small x limit when the extension is small or the forces are small it should come out like a hooky in spring and that it has. But, it is quite generally you can write down the extension, if the forces become large, then this is no longer a linear relationship it is now given by this tan hyperbolic ok. And if you plot that if you plot this z; if you plot this relative extension.

If you plot this relative extension as a function of this force again you will get this sort of linear saturating to 1; this is not a relative extension it will saturate; this is just this curve with the axis is flipped. So, this is precisely the sort of behavior that you see in these experiments for this DNA double strand pulling. So, this is precisely the sort of behavior that you see in these experiments.

And if u in for example, if you know the force and you know the temperature at which you are doing this experiment, this tells you precisely what is the coon length for this particular polymer in order for in order to model this polymer as a freely jointed chain as a 2 dimensional random. Yes. So, for example, in that AFM setup its one end was fixed and the other end was being pulled with a particular force right..

For example, if I look at this setup over here this end was fixed and I was pulling on that end. You could do different things; you could also maybe pull both ends and so on depending on what experiment you have done. Depending on the setup you might need to alter the calculation of it but for a setup like this would be an appropriate calculation to do. Actually so, for example, one thing is I did this for a 1D polymer, you can actually do this for a three dimensional polymer, do exactly this similar sort of a thing; because in reality the DNA that you are pulling is a 3D polymer.

So, let s say I if I do a 3D polymer and I should get something very similar of course, I will get a different expression than you can see what it is ok.

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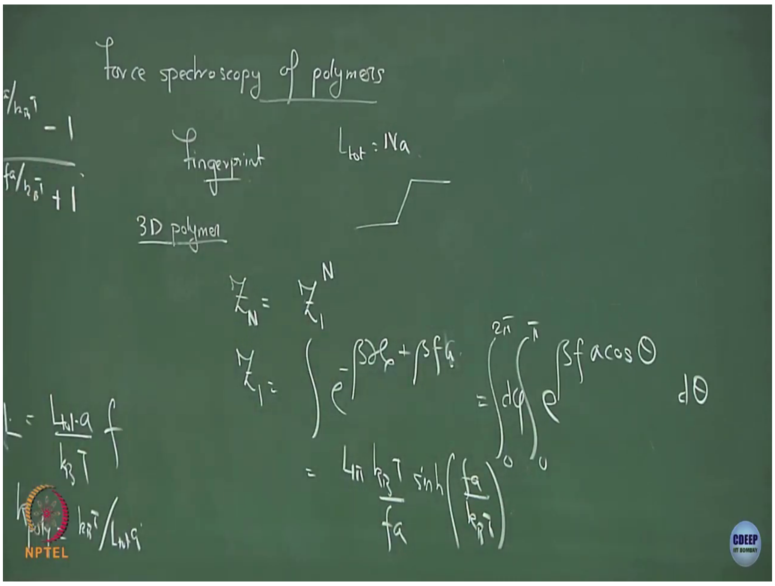

So, let us say I have a polymer again this is of N link. So, the l total is N times a and so on. And again I work for this ideal polymer chain where there are no interactions between the segments. And these there are no correlations between the segments; which means that each monomer sort of does its own thing independent of the other right independent of any others.

So, if I want to write down the partition function Z N for independent links like a freely jointed chain what I will have is that this is nothing but, the single particle partition function raised to the power of N right. This is the non interacting case exactly like it over done an ideal gas. I can calculate this Z 1 I can calculate this Z 1; this is nothing but integral. Let us say e to the power of minus beta h plus beta j dot x so, fL so on.

Again this is ideal so, I do not know I wrote the h there is no h in this case. So, this is nothing but e to the power of beta f times a cos theta, if we not write l here let me write a here because I am working for the single part single link partition function ok. And this I would then need to integrate over all possible confirmations. So, d phi 0 to 2 pi d theta 0 to pi if I do this integration what I will get is a 4 pi k B T by f a sine hyperbolic fa by k B T.

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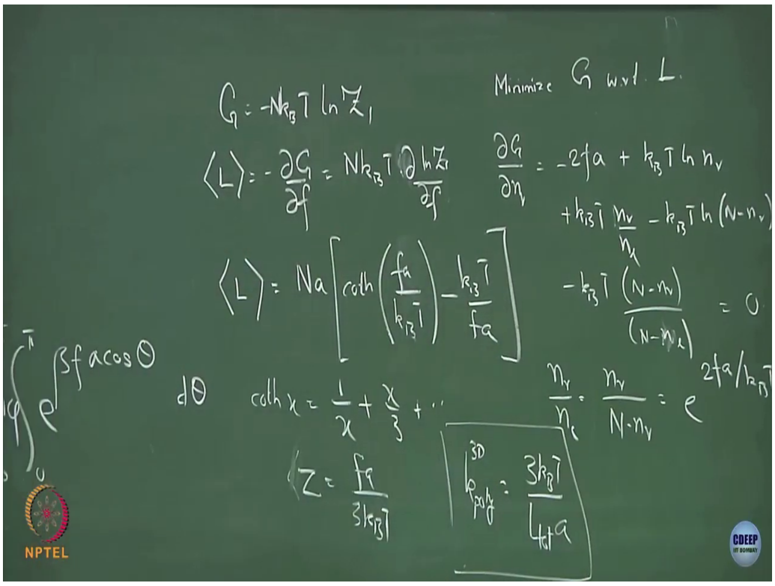

Minimize G w.r.t. L.

$$G = -Nk_BT\ln Z_1$$

$$\langle L\rangle = -\frac{\partial G}{\partial f} = Nk_BT\frac{\partial \ln Z_1}{\partial f}$$

$$\langle L\rangle = Na\left[\coth\left(\frac{fa}{k_BT}\right) - \frac{k_BT}{fa}\right]$$

$$\int_0^{\pi} e^{\beta f a\cos\theta}\, d\theta$$

$$\coth x = \frac{1}{x} + \frac{x}{3} + \cdots$$

$$Z = \frac{fa}{3k_BT}$$

$$\boxed{k^{3D}_{poly} = \frac{3k_BT}{L_{tot}a}}$$

$$\frac{\partial G}{\partial n_v} = -2fa + k_BT\ln n_v + k_BT\frac{n_v}{n_v} - k_BT\ln(N-n_v) - k_BT\frac{(N-n_v)}{(N-n_v)} = 0$$

$$\frac{n_v}{n_c} = \frac{n_v}{N-n_v} = e^{2fa/k_BT}$$

So, that is my single particle partition function Z N is just the Z 1 to the power of N and the Gibbs free energy G is nothing but minus k B T log of Z N. So, log of minus N k B T log of Z 1 right and this average L the extension is nothing but minus del G by del f. So, this is nothing, but N k B T N k B T log N k B T del of logs Z 1 by f. And again you can take, you can do this differentiation; I am not doing it explicitly just write down the result.

So, if you do this what we will get is, this extension is nothing but N times a cot hyperbolic fa by k B T minus k B T by fa. So, the relative extension Z is nothing but this term inside the brackets cot hyperbolic of fa by k B T minus k B T by fa. You can again check that for consistency in that if fa is very if this argument is very small, it should give me back a hooky in spring.

So, you can expand cot hyperbolic x for small x and for small x this is cot hyperbolic x is 1 by x plus x by 3 plus (Refer Time: 19:11). So, if you do this, the 1 by x will exactly cancel this term. So, the leading order term will be x by 3; so, in the small x limit what I will get is that this relative extension; let me just write is nothing but x by 3. So, fa by 3 k B T; which means that the spring constant of this 3 dimensional polymer spring constant of this 3 dimensional polymer is nothing, but 3 k B T by L total times a which is again what we got last class.

But now, we have the full force extension relationship for a 3 dimensional polymer, this is how it would look. And again if you plot this relative extension L by N a as a function of the force; you will get a curve which looks exactly like this like you seen in these experiments.