Basics of Fluorescence Spectroscopy
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Lecture - 33

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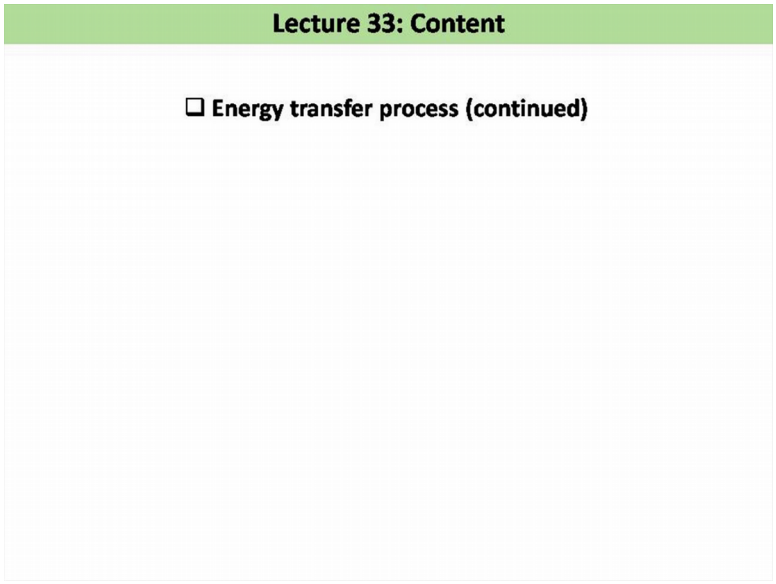

Welcome to lecture number 33. In the last lecture we have seen 2 equations: one is a relation between the efficiency of energy transfer with the distance r, that r is the distance between the donor and accepter. And another equation what we have seen, these efficiency of energy transfer that is coming out either from the decrease in the Fluorescence intensity of the donor in absence, in presence of the accepter or the decrease in the life time of the donor in presence of the accepter.

And we also have seen that if you in a plot this E the efficiency of the energy transfer as a function of r, the distance between the donor and accepter that shows a typical sigma type of curve. So, as it is evident from here, let me draw and then will discuss.

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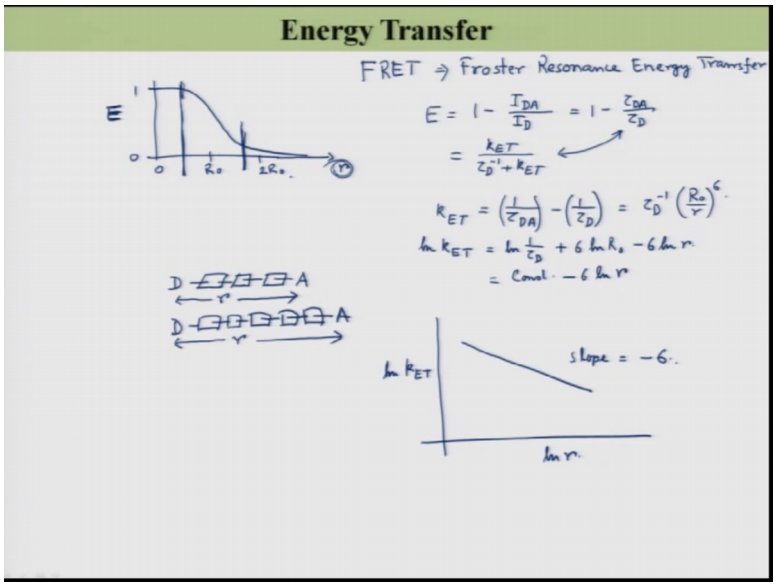

So, that curve what he saw is something like this. And here is my 1 and here is my 0 if I plot E and here is your r and here is 0, R 0 here is your 2 r 0.

So, see this efficiency at of energy transfer this capital E, is very sensitive in this region, very sensitive in this region, right? Very sensitive in this region little bit of change of r will change this E drastically right. So, now, I can tell you on the other hand that if you now can experimentally determine this E, you will get some idea of this r, what is the value of r? If you know this capital R 0 and this capital R 0 I said is Forster distance. And this Forster distance for a particular donor accepter theory is fixed.

So, situation is something like this, right? I have to determine these capital E the efficiency of energy transfer experimentally and then R c though, the capital R c though has to be determined before hint then for a giving measurement if for the given value of capital E it is easy for me to comment about the what is the distance between the donor and accepter, clear. That is why these energy transfers these energy transfer process are widely used in biology and this is also referred to as the molecular ruler.

So, using molecule you can measure a distance right. So, is refers to as molecular ruler. Also one thing you should mention over here that this is energy transfer that is for sure as we discussing from the last 2 lectures. And this energy transfer is possible only when the emission of the donor is in resonance with the absorption of this accepter. The energy should match otherwise it is not possible that is what I said, right? And you have seen. In

this case the interaction between the donor accepter is by the columbic interaction. And which was introduced by Forster for the first time. So, this process is known as Forster resonance energy transfer. The short form of this spread is very popular among the biologist. Because this is a procedure by which one can determine is very small distance and it is very sensitive to the distance, I mean that efficiency of energy transfer is very sensitive.

To the distance over this region as I said over this region it is extremely sensitive. So, a small change in the distance will D if a large change in the E. So, u can use this E to measure a very nicely or very accurately the distance between the donor and accepter. So, this energy transfer is actually call this fret which is nothing but Forster resonance energy transfer. And, as I said earlier that this E can be measured experimentally.

So, this is equal to 1 over I DA, what is I DA? Intensity of donor in presence of accepter divided by id again same as 1 minus tau DA divided by tau D again same. A life time of the donor in presence of accepter, and life time of the donor this tau D is life time of donor in absence of accepter, then you can determine these and I also told u that this is equal to K ET divided by tau D inverse plus K ET. So, I can simply equate these with this right. So, if you do. So, what you will get ultimately is K ET is equal to 1 over tau DA minus 1 over tau d, right? Just after simplification.

So, the result will come like this way, right? Now experimentally you measure this guy E, right? Or experimentally E measured this tau experimentally E measured this tau the life time of donor in presence of accepter and life time of donor in absence of accepter. And immediately you will get this value of K ET and this value of K ET is nothing but tau D inverse R 0 by r to the power 6 and from here you will be able to calculate; what is this r what is the distance between the donor and the accepter, right. Now being said all those things, right? This is a theory nice theory by Forster somebody has to prove it that really it is working that once I change the distance which in the donor accepter the efficiency is really changing like that way, that is in the order of to the power 6 remember, that r to the power 6.

So, somebody has to prove it experimentally, right? And that that thing was done by taking a molecule like this way, this molecule looks like this. Something over here something over here some molecular the thing frequency connected with each other. And

you put D here and you put a here, right? This is actually a typical polypeptide change, right with little bit rigidity. So, that you know the distance here because your synthesizing, right. So, you know the distance over here. Now if you look at these equations, these equation K ET equal to tau D inverse R 0 by r to the part 6, right? And you take l n both side I can write l n K ET is equal to, right? You understand. So, l n 1 over tau D because this is tau D inverse I took the l n plus 6 l n R 0 and then minus 6 l n r.

So, this is equal to this is a constant, l n 1 over tau D is a constant. 6 l n R 0 is a constant because R 0 is a constant; so some constant minus 6 l n r. It means that for such a molecule if you can vary this distance, right? Suppose you have prepared all like this and then you have prepared another like this and you know in organic chemistry senses is, somehow you know; what is this r, right? And you can vary r as per your desire and at each molecule you will going to measure this K ET. How you measure this K ET? Is easy you just simply measure this life time over here as you seen here.

So, you just measure this life time of this molecule, right? And you measure the life time of only the donor without this accepter then you measure this K ET. And then you plot this l n K ET as a function of r, right? For several such kind of molecule you should not change the donor accepter and that was done, right? To prove these Forster resonance energy transfer whether it is valid or not.

So, what you simply do? You simply plot this K ET sorry, l n K ET, right and versus l n r. Because you know all those things and it was proved that the slope in this case is equal to a minus 6, the slope is equal to minus 6. That is it, is already proved, right? That whatever the Forster resonance energy transfer is actually operational, is actually operational. I will tell you one thing, when the people have studied this type of Forster resonance energy transfer in case were the r value, the small r value too small people have seen that this is deviating from this type of equation deviating from r to the power 6 dependence.

And there the exchange interaction is more favorable compare to these columbic interactions between the donor and accepter. These r to the power 6 terms comes in because of the columbic interaction the orbital’s are not over lapping with each other, but if it is So, why should the interact with this columbic interaction?

The electron can jump from one orbital to another orbital is not necessary that columbic interaction will takes place in this case. And that is the case of the electron transfer that is the case of the electron real physical electron transfer. And here the because of the columbic interaction the 2 are for effort and the interaction between 2 are taking place at a distance this is because of the columbic interaction So they are different. Now as I informed you briefly, let me show you that how this is help full as a molecular ruler right. So, typically I use a example of a protein.

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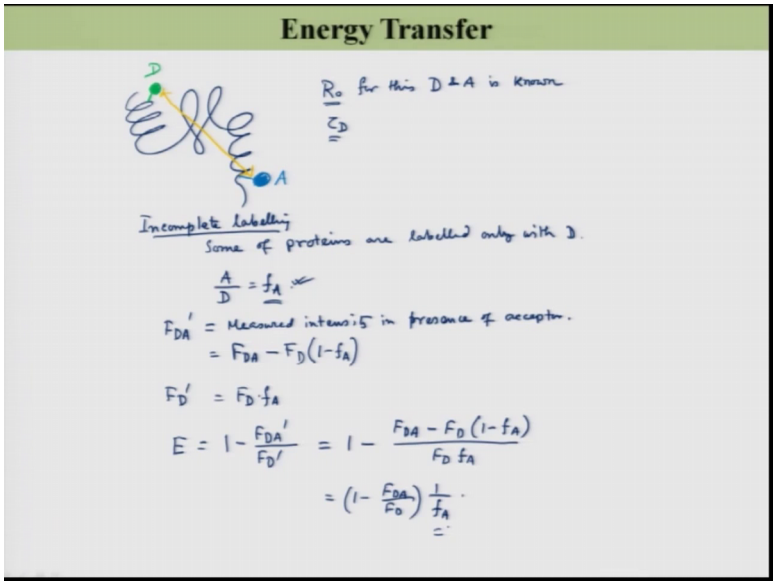

So, let me take a protein and draw it like this way, like this way. And somehow you have some mechanism to level this protein with a donor and the accepter. How? That is the different story and I am not going to discuss that in this lecture, but it is possible.

So, let us assume that you have attached one donor at this location and somehow you have attached another accepter at this location. And if you take this donor and accepter, right? This particular D and A you will be able to calculate that capital R zero; that means, your capital R 0 known, capital R 0 for these D and A is known. If not known you measure it. Because you know the form, right? Although I have not written explicitly, but any standard book you will get this form right. So, you know this R 0. So, what you have to do now? And you also know this tau D, right? And you also know the tau D, what is tau D? Tau D is the life time of this donor in absence of the accepter. Well that means, donor is alone, right? You have not level this a in this version now simply

what you can do we can just measure the tau D A, right? Once you measure the tau DA immediately you will get 1 over tau DA minus 1 over tau is equal to k ET. And K ET equal to tau D inverse into R 0 by r to the power 6.

And from that you can you can immediately tell what is the distance between this t to a tick in immediate, later no problem. Now consider that this protein is undergoing some structural change, some structural change. Because you have heated the protein or you did something to this protein and you change the temperature you change the you decrease the pH or something like that

So, which is the protein structure we will destroy. That is called the unfolding of the protein or denaturation of the protein. And as the protein will denature it is expected that the distance between the donor and accepter will increase right. So, you can measure the distance between D and A which will be a measure of the extent of unfolding of the protein.

So, if you now plot the distant between D and A as a function of outside agent which will damage the protein let say for temperature then you will get a clear idea how the protein is denaturating starting from it is native state to the denatures it. So, this is very nice example of or very nice use of these Forster resonance energy transfer fret. And where it is also called this molecular ruler, but here you notice one thing. During the calculation of this capital R 0 I said the value of kappa square is equal to 2 by 3 and I immediately took this 2 by 3 inside my constant.

So, that capital R 0 is a constant. What you really think that once this donor is attached to a part of a protein and then accepter is also attached to another part of the protein? They could have at under orientation; that means, the relative orientation of the donor compare to the accepter could be anything is not possible; that means, probably it is not possible to use the value of kappa square is equal to 2 by 3. And the last few decades people are actually trying to understand that what is the specific role of these relative orientation when we will going to use this fret in bio molecular system. Suppose u have labeled some protein with only donor and some protein with only donor and some protein with only accepter and some protein with both the donor and accepter.

So, this is the typical case of incomplete labeling. So, in case of incomplete labeling what happened is. So, what happens is some of protein are leveled only with D no A,

right? No A in that case what will happen in that case whatever the fluorescence intensity your expect to be decreased for a specific value of E, that much will not decrease because some proteins have only D. Now in presence of A I mean you thought that fluorescence will be half, but for some protein they do not have A itself. So, the those protein those protein those has D and A together their process intensity decrease to 50 percent, but those does not have this A, but only D their florescence it will not going to decrease 50 percent. So, that is the problem.

So, I have to ensure that the protein which has D must as A, but that is very difficult for the censure. If I somehow know what is the ratio of proteins that has D, but does not has this A if I know this ratio then probably it is I can take into account. So, let us tell that the ratio of A by D is equal to let say f A. So, then I can write f DA prime which is equal to measured intensity in presence of accepter which should be equal to f DA minus the quantity of florescence intensity originated from those protein which as only the donor not accepted right. So, that is f D that is my original florescence intensity multiplied by 1 minus that factor f A. So, I know this f A then only I can write this right. So, and f D prime should be equal to f D into f A that is it.

So, that efficiency of energy transfer should be written as 1 minus f DA prime by f D prime, right? I do not measured f DA prime f D prime. What I measure is, I measure f D and f D that is it. I do not know; what is f Di prime f D prime, but somehow if I know this f A this quantity then only is possible for me. So, this can be written as 1 minus f DA minus f D to 1 minus f A divided by f D to f A which is equal to 1 minus f DA by f D to upon over f A. So, you see I get this efficiency in this form right.

So, if you measured this efficiency just by this f DA by f D then that will be a wrong answer right. So, you need to multiply with this factor on of our f A in this case. So, let us finish over here and we will continue our discussion on the next class.

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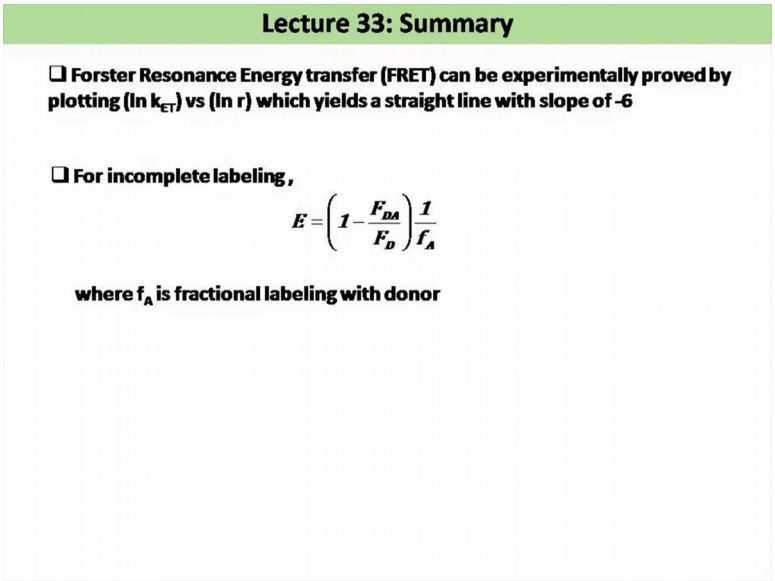

Thank you very much.