

Basics of Fluorescence Spectroscopy
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Lecture - 32

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Lecture 32: Contents

□ **Energy transfer process (continued)**

In the last lecture we were discussing about the Energy Transfer, and specifically we were discussing about the non-radiative energy transfer process.

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Energy Transfer

$D^* + A \rightarrow D + A^*$

$$k_{ET} = \frac{\kappa^L}{2D^2 r^6} [\quad]$$

$r =$ distance between donor and acceptor.
 $\kappa^L =$ Orientation factor.

$$\kappa^2 = (\cos \theta_T - 3 \cos \theta_D \cos \theta_A)^2$$

Case I : $\left. \begin{matrix} \theta_A = 0 \\ \theta_D = 0 \\ \theta_T = 0 \end{matrix} \right\} \kappa^2 = 4$

Case II : $\left. \begin{matrix} \theta_A = 90 \\ \theta_D = 90 \\ \theta_T = 0 \end{matrix} \right\} \kappa^2 = 1$

Case III : $\left. \begin{matrix} \theta_A = 90 \\ \theta_D = 90 \\ \theta_T = 90 \end{matrix} \right\} \kappa^2 = 0$

So, in the non-radiative energy transfer process what I showed you that D star is present and which is interacting with A and this energy is transferred from D to A star, as a result D is getting deactivated and A is now in the excited state. So, if I now tell you the this molecule D star as the one electron is over here another is over here, and this A is present A is in the ground state then the situation is that these two a and D are actually interacting over a distance and which is converting this a this D as deactivated and a is now in the excited state.

And I said that for this dipole interaction. The interaction which is responsible is a columbic type of interaction and then again I showed you that equation here this k_{ET} can be written as this κ^2 divided by τD^6 alright and then some constant like this way and I said that this κ^2 is where this r equal to distance between donor and acceptor and this this should be τ (Refer Time: 02:23) τD^6 and then κ^2 is my orientation factor.

This κ^2 depends on the relative orientation of the donor and the acceptor, and if you look at this specific form of this κ^2 let me draw this donor and acceptor this dipoles like this way. Let us say this is the donor dipole oriented in this fashion and this is this acceptor dipole is oriented in this fashion and this is the distance between donor and the acceptor. So, this is my D, this is my A, now if you extend this line the line connecting the D and A and then you measure this angle the angle between this line and the acceptor and in this angle let us say is θ_A and this angle similarly is let us say θ_D .

And if you make a plane with this donor dipole and this line the line connecting donor and acceptor and donor dipole, with these if you make a plane and the acceptor dipole and the line connecting the donor acceptor with these if you make another plane like this then the angle between these two plane let us say termed it as θ_T . So, this κ^2 actually depends on these 3 θ values with this form as $\kappa^2 \cos^2 \theta_T \sin^2 \theta_D \sin^2 \theta_A$ and whole square. So, this is my expression for the κ^2 .

Let me give you three different examples alright. So, let us say case 1, let us say this is my donor dipole and this is my acceptor dipole right and the distance between them is r right. So, that is my distance, but that κ^2 will not going to depends on that

distance it will only depend on that orientation, but total k ET value will (Refer Time: 05:54) obviously, depend on the distance. And you note here that dependency of this distance is one over r to the power 6 little change in r will change the k ET value in enormously right.

I will come later I will tell all those things in detail. So, in this case if I tell you that what is the value of theta A, what is the value of theta D and what is the value of theta t, what will be your answer theta A is equal to 0 theta D equal to 0, theta T equal to 0. So, for these values of theta kappa squared will be equal to $\cos^2 \sin^2 0 - 3 \cos 0 \cos 0$ right. So, this will be equal to $1 - 3$; so minus 2 square. So, is equal to 4. I will take another (Refer Time: 06:58) case two this is my acceptor same distance r (Refer Time: 07:11) this is my donor and this is my acceptor for this case two I will also have different values of theta A theta D theta T. So, theta A is equal to 90, theta D is equal to 90 and theta T here will be equal to 0. So, in this square kappa squared will be equal to 1 right.

Let me show you another case which is case 3, in this case let me take my dipoles this donor and acceptor like one is like this, this is my donor and this is r right and this is my acceptor. Such way that theta A, the theta A is equal to 90 right theta D is also 90 and the angle between these two is also 90 degree right. And this will going to give me kappa squared equal to $\cos^2 \sin^2 90 - 3 \cos \sin 90 \cos \sin 90$ right. So, this is going to be equal to 0. So, you see depending on the orientation of these two dipole mu D and mu A.

The value of kappa squared actually varying a lot starting from 4 to 0 and that is the problem. Suppose you now you think of a situation you have (Refer Time: 09:19) the donors right. And the how these donor molecules will orient in solution that will not going to depend on anything, because as we have already seen in the fluorescence (Refer Time: 09:33) on during our discussion on fluorescence that the orientation of these molecules in a solution is random, that is called the isotropic orientation.

That means the donor molecules will orient in all possible conditions. Similarly the acceptor will also orient randomly in all possible orientation; that means, the theta A, theta D, theta T the values of theta A, theta D, theta T could be anything right. So, because of this isotropic orientation of the donor and the acceptor for solution, but for a rigid system like a biomolecules let; I will talk to the I will talk about this biomolecule later on, but it is for sure that for solution state that the orientations of the donors and

accepters are random and is a isotropic emission; so better to have a case four where I will take these randomizations of the donor and its acceptor.

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Energy Transfer

Case - IV : Donor and acceptor are oriented randomly. (isotropic orientation).

$$\kappa^2 = \frac{\int_0^\pi \int_0^{2\pi} (\cos\theta_T - 3\cos\theta_D\cos\theta_A)^2 d\theta_T d\theta_D d\theta_A}{\int_0^\pi \int_0^{2\pi} d\theta_T d\theta_D d\theta_A} = \frac{2}{3}$$

$$k_{ET} = \frac{\kappa^2}{\tau_D^0 r^6} \left[\int_0^\infty J(\nu) I(\nu) d\nu \right]$$

$$= \frac{C R_0^6}{\tau_D^0 r^6}$$

← For spectral overlap integral. This is a constant for a definite pair of donor and acceptor.

$$k_{ET} = \frac{1}{\tau_D^0} \left(\frac{R_0}{r} \right)^6 ; R_0 = \text{Forster distance.}$$

Efficiency of energy transfer.

$$E(t) = \frac{k_{ET}(r)}{\tau_D^{-1} + k_{ET}(r)} = \frac{\tau_D^{-1} \left(\frac{R_0}{r} \right)^6}{\tau_D^{-1} + \tau_D^{-1} \left(\frac{R_0}{r} \right)^6} ; \tau_D = \frac{1}{k_r + k_{nr}}$$

$$= \frac{1}{1 + \left(\frac{r}{R_0} \right)^6}$$

So, for this case 4 what I have is the donor and acceptor are oriented randomly, this are isotropic right orientation, in this case kappa square is retained as average value right. So, I will integrate 0 to pi cos sin theta T minus 3 cos sin theta D cos sin theta A square D theta T, D theta D, D theta A and (Refer Time: 11:56) normalize it with 0 to (Refer Time: 12:00) theta D theta T theta A. And if you now the value of this integral is very simple which come out comes out to be 2 by 3. Now you remember our original location this k ET I had written this as kappa squared by tau D r to the power 6 then I said this is a constant right. So, this is (Refer Time: 12:38) 0.

Now, if I consider this random orientation of this donor and acceptor then this quantity is also will come inside this constant. So, I will trite this as A constant divided by tau D 0 r to the power 6, but remember that this constant has spectral overlap integral within it means this is a constant for a definite pair of donor and acceptor. So, this is a constant for a definite pair of donor and acceptor and that constant is written as R capital R 0 to the power 6, that I can do that is mine (Refer Time: 14:09) my desire right (Refer Time: 14:10) capital R 0 to the power 6.

And if you know this overlap integral between this donor and acceptor and from the expression of this quantity over here you will be able to calculate the value of R 0 and

that value of R_0 is fixed for a definite donor and acceptor. So, the final equation will be k_{ET} is equal to $1 / \tau_{D_0} R_0^6$ and that particular constant R_0 is known as Forster distance, that is known as Forster distance.

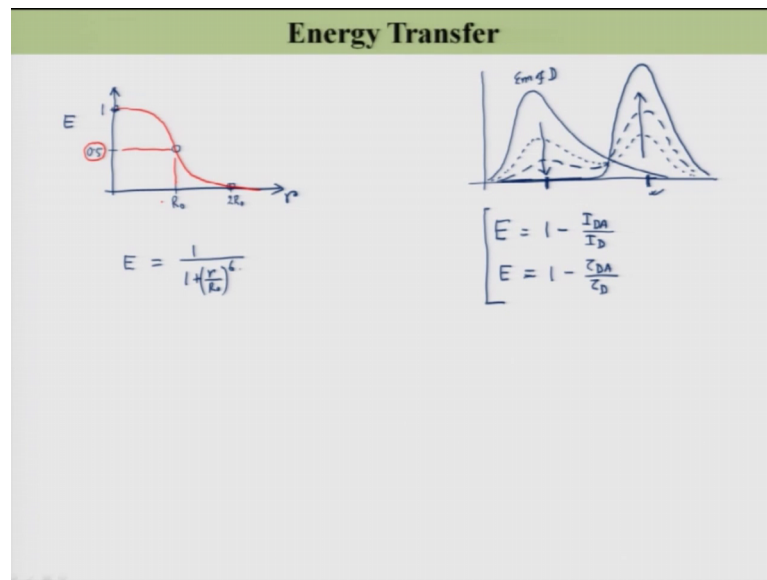
Now let me define a quantity which is called the efficiency of energy transfer denoted by capital E . Right let me define and then I will explain what is that. Efficiency of energy transfer is denoted by capital E which is equal to the rate of deactivation from the excited state to the ground state right the rate constant that is leading to the energy transfer divided by the total right like similarly for the quantum yield I divided by $k_e r$ by $k_e r$ plus k_{NR} for fluorescence quenching what do we had that for the quantum yield that is $k_e r$ by $k_e r$ plus k_{NR} plus k_q into q .

So, in this case another path way which is non-radiative path way; that means, energy is taking out by the system this k_{ET} . So, in this case the efficiency should be k_{ET} divided by the total rate constant right and total rate constant means inverse of τ_{D_0} ; that means, this k_r plus k_{NR} . And then that another rate constant which is responsible for the deactivation is k_{ET} rate constant of the energy transfer. So, this efficiency of energy transfer can be easily retained as k_{ET} divided by τ_{D_0} . So, in this τ_{D_0} I am going to have this k_r plus k_{NR} right because you remember τ_{D_0} (Refer Time: 17:04) τ_{D_0} equal to $1 / (k_r + k_{NR})$.

So, I am going to have these all the time this plus k_{ET} right and you also please remember that this k_{ET} is a function of r small r this is a distance between the donor and acceptor and that distance dependence is very high is r to the power 6. So, I better write here as a function of r this is a function of r . So, this is also function of r . So, efficiency is also a function of r . So, then I can just simply put the expression of k_{ET} r in this equation right. So, if you do that. So, then this will be $\tau_{D_0}^{-1} / (\tau_{D_0}^{-1} + \tau_{D_0}^{-1} R_0^6)$, right from here divided by $\tau_{D_0}^{-1} + \tau_{D_0}^{-1} R_0^6$.

So, if you just simplify this expression what you will going to see is one divided by $1 + R_0^6 / r^6$ you will get such kind of expression right. So, efficiency of energy transfer is something like this, as I told u earlier that if this is my emission spectra D in presence of acceptor.

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There is a decrease in the intensity and there is an increase in the intensity of another wavelength just like this. So, here the intensity will decrease and here the intensity will increase. If you monitor the fluorescence intensity at this wavelength and fluorescence intensity at this wavelength then with increase in (Refer Time: 19:59) of this acceptor concentration. That means, the average distance between the donor and acceptor will change you will get a reduction in the fluorescence intensity and in wavelength the fluorescence intensity will increase.

So, that is also in some other words must be corrected with the efficiency of the energy transfer, suppose you have added some molecule and nothing happens alright the fluorescence intensity remains the same as without the presence of this acceptor that means nothing happens then the efficiency of energy transfer if I now may say like that then the efficiency should be 0 that is it. And let us say for example: you have added this acceptor and the whole complete quenching of this fluorescence right there is nothing it is like this 0 over here and only this guy is like this it means the efficiency is 100 percent where the efficiency is equal to 1. So, if I now take this efficiency in a scale between 0 and 1. So, I can tell this efficiency in terms of the fluorescence intensity in presence of acceptor and in absence of acceptor that is it.

So, if I this efficiency E is typically retained as one minus I_{DA} by I_D right. So, you see here this efficiency when the I_{DA} ; that means, the intensity of donor in presence of

accepter is same as I_D ; that means, there is no change in the emission intensity. So, then $I_D A$ by I_D equal to $1 - 1$ equal to 0; that means, the energy transfer is completely inefficient I mean 0 nothing. Now you consider that after addition of this acceptor the fluorescence intensity became 0 I mean from a some value it (Refer Time: 22:11) could be 100 from 10 to 0 or 1000 to 0, but it (Refer Time: 22:12) whatever it is from there it is 0; that means, this 0 by something is $0 - 0$ equal to 1. So, then the efficiency is one; that means, (Refer Time: 22:23) fully efficient energy transfer.

Similarly, this efficiency also can be retained in terms of the lifetime; in case of energy transfer this lifetime of this species will also change, because of the creation of this additional pathway. What is that additional pathway? That I wrote in just few minutes back as k_{ET} that is my additional pathway that is why this when I have calculated the efficiency right I have retained this k_{ET} divided by the total rate constant and that is a rate constant. So, the fluorescence lifetime will also change. That means, I can write also this efficiency in terms of $1 - \tau_D A$ divided by τ_D . So, here this τ_D is the lifetime of the donor in absence of this acceptor and $\tau_D A$ is a lifetime of the donor in presence of acceptor.

Now, you (Refer Time: 23:21) you can say that if I increase the acceptor alright then; obviously, this (Refer Time: 23:27) will be different, but the reason is not because you have more acceptor the reason is that the average distance between the donor and acceptor will decrease we have those one donor and 100 acceptor then from donor to the acceptor distance average distance is something, if you have one million then average distance will be much smaller. So, that I why you are going to have it because the only thing which is depend here is the distance right and (Refer Time: 23:52) for a definite donor acceptor pair that $r_{capital R 0}$ is a constant.

So, telling this now I can simply do one thing I can (Refer Time: 24:09) a plot like this where this y axis is my E and this x axis is my r and now I can comment on that right what will be the value of E that efficiency as I change my r value right that that that will automatically come right. So, (Refer Time: 24:38) what equation I already have? I have this equation in involving the efficiency of the energy transfer as well as the r the distance between the donor and acceptor.

Here by this way I will going to calculate this efficiency right and then here capital E equal to 1 over 1 plus r by R 0 to the power 6 with this I will plot this right. So, let us take a value when r equal to 0 that small r equal to 0 here is my r when this small r equal to 0. So, it is over here. So, 1 plus 0 to the power 6 and that is inverse. So, this is equal to 1. So, this value will be equal to 1.

And when r equal to R 0 right let us say take a point over here, where this value of r equal to R 0 right in that case this R 0 by R 0; that means, 1, 1 divided by 2 equal to 0.5. So, here I will get 0.5 here is my point and here is my another point and when r equal to twice R 0 then this twice R 0 divided by R 0 that is 2 to the power 6, that is 2 to the power 6 that is 64 that 1 divided by 65 that will come about 0.015. So, it is almost over her almost over here and in between if you plot the value will come what we will going to see the plot will something like this right. So, from here I can at least tell that when the efficiency of energy transfer is just 0.5 that r is equal to capital R 0 which is my first (Refer Time: 26:55) distance right. So, let us finish here and we will continue our discussion on the next day.

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Lecture 32: Summary

- Orientation factor, $\kappa^2 = (\cos\theta_r - 3\cos\theta_d\cos\theta_a)^2$
- For an isotropic orientation of donor and acceptor in solution, $\kappa^2 = \frac{2}{3}$
- $k_{ET} = \frac{1}{\tau_D} \left(\frac{R_0}{r} \right)^6$
 R_0 is called Forster distance which is constant for a definite pair of the donor and the acceptor
- Efficiency of energy transfer, $E = \frac{1}{1 + \left(\frac{r}{R_0} \right)^6}$; $E = 1 - \frac{I_{DA}}{I_D} = 1 - \frac{\tau_{DA}}{\tau_D}$
- Forster distance is the distance between the donor and the acceptor for which efficiency of energy transfer is 0.5

Thank you very much.