Basics of Fluorescence Spectroscopy
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Lecture - 31

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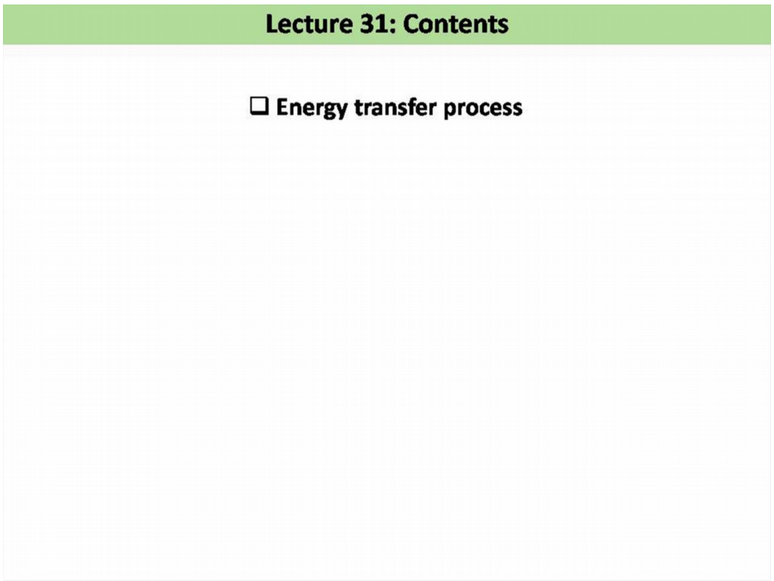

Lecture 31: Contents

☐ **Energy transfer process**

Welcome back to the lecture number 31. Today we will going to discuss a defined topic which is called the Energy Transfer.

As I told you briefly during our discussion on fluorescence quenching like this, this is the broad topic of fluorescence quenching is the quenching of the fluorescence intensity by the presence of some other molecule, which we termed as the quencher molecule. And the reasons for the fluorescence quenching are of many faults. For example, the electron transfer is one of them. So, when a molecules is being excited to the excited state and the fluorescence comes from the excited state of the molecule.

Now, if you have one electrons deficient molecule present nearby to this fluoropore which is in the excited state. That the other molecule can donate one electron to the homo of that fluoropore which makes the fluoropore to have a filled homo and the fluorescence will be deactivated immediately. Or in other case it can take the excited electron from this fluoropore when the other molecule is electron deficient, right.

Similarly, this energy transfer is another case of the fluorescence quenching. I can describe this energy transfer as one of the case of the fluorescence quenching. For example, like if you add right some kind of molecule then the emission intensity will decrease of the original fluoropore. That excess of energy could be transferred to the other molecule. And that may create a new emission band of the other molecule. Let me write point by point.

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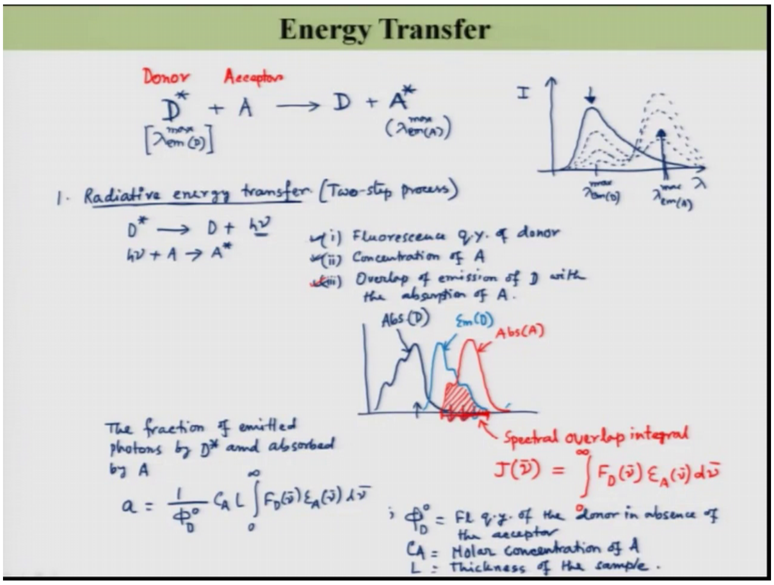

Energy Transfer

Donor Acceptor

$$\underset{[\lambda^{max}_{em(D)}]}{D^*} + A \longrightarrow D + \underset{(\lambda^{max}_{em(A)})}{A^*}$$

1. Radiative energy transfer (Two-step process)

$D^* \longrightarrow D + h\nu$

$h\nu + A \rightarrow A^*$

(i) Fluorescence q.y. of donor

(ii) Concentration of A

(iii) Overlap of emission of D with the absorption of A.

Spectral overlap integral

$$J(\bar{\nu}) = \int_0^\infty F_D(\bar{\nu})\,\varepsilon_A(\bar{\nu})\,d\bar{\nu}$$

The fraction of emitted photons by D^* and absorbed by A

$$a = \frac{1}{\Phi_D^0} C_A L \int_0^\infty F_D(\bar{\nu})\,\varepsilon_A(\bar{\nu})\,d\bar{\nu}$$

; Φ_D^0 = Fl q.y. of the donor in absence of the acceptor

C_A = Molar concentration of A

L = Thickness of the sample.

So, what I was trying to tell you is that- you have a molecule I name this molecule as D, and it is these molecule is in the excited state. So, it is D star. So, in absence of any other thing these molecule will going to give you emission or fluorescence, and you can record the fluorescence intensity as a function of wavelength the emission wavelength which is nothing but the fluorescence spectrum or emission spectrum of these particular molecule. Now consider you have another molecule present in the solution that molecule I write as A, and the result is that these molecule is being deactivated and simultaneously these other molecules is getting excited.

Now, here this has a different emission maxima let us say this emission lambda emission maxima or D, but this molecule will have a different emission maxima lambda, emission maxima of A right. So, I can have. So, as a result what you will see you will see the fluorescence. Now if I may plot right this is my fluorescence intensity, I and then this is my wavelength lambda.

So, initially when there was no a molecule what you would have seen is that this is your fluorescence intensity and this corresponding maxima is your lambda, emission, maxima of d as you are adding this a molecule then these fluorescence intensity will decrease, but a new fluorescence will appear like this way. If you add more a then this fluorescence will further decrease and then this new fluorescence intensity will be much more and if you had further it will be something like this.

So, this new emission maxima is because of the A not because of the D. So, this is lambda emission maxima of A. So, now, if you look at this wavelength right you will see that fluorescence is quenching, but in this particular case here at this particular wavelength the fluorescence is actually increasing also since it is special type of thing right. So, what just looking at I can simply tell that here this molecule, this molecule means if this D star, the energy from D star is getting transferred to A. So, that as a result that d is getting deactivated not D is in the ground state and A is now in the excited state. So, by denoting it to star; that means, this D star is my energy donor and this a is my energy accepter.

So, simply we write this as donor and this as donor and this as accepter. Now question is that how the energy is getting transferred right. So, I can think of a simple path way by which the energy is transferred let me write this as D star is getting converted to the ground state of the donor, and plus the emitted photon was the h mu. So, I can write D 2 D plus h mu I can simply write that, and then I can write these h mu is somehow being absorbed by this a molecule to make this A from the ground state to the excited state.

So, I have first radiative emission of this photon this photon and this photon is being absorbed again by this accepter A. So, this this is a two step process and this is known as radiative energy transfer. So, I can write this as case one radiative energy transfer and this is a two step process. Now just looking at this mechanism as (Refer Time: 07:41) as I have written here it is evident that if the donor quantum yield is high. That means, right the non-radiative rate constant is much higher than the non-radiative rate constant.

So, this process must depend on the quantum yield of the donor. Again if you look at this you see that this emitted photon has to be absorbed by this a and that clearly tells me if I have more A present nearby the D then this process right then these process is are more feasible. So, it also directly proportional to the concentration of A molecules present in the solution. If you again look at this diagram automatically you understand that the emission wavelength of this donor must be very similar to the absorption of this accepter. So, I can write these three different conditions over here.

So, the condition number one is the fluorescence quantum yield of donor, two I can write the concentration a and three I can write that overlap of emission say like these are the three condition if the fluorescence quantum yield is more than; obviously, the emitted photons will be more and then concentration of A is more than the possibility of the emitted photon will be absorbed this is also more and then the third is the overlap of emission of D with the absorption of A. If these three thing satisfy then efficiency of this energy transfer will increase right let me this in the point number one and point number two is easy to understand, but let me elaborate this point number three.

For example: let us say this is the absorption spectrum. Now let me draw first, let us say the absorption spectrum of the donor. So, I will just write here abs of donor depending on I mean suppose you are exciting at the maxima. So, here is your lambda excitation alright then you will get the corresponding emission spectrum of this, let us say this is your emission spectrum of the donor alright. You see I have drawn as a mirror image just because the I just assumed that here the in the excited state there is no structure change in this molecule. So, mirror image rules holds good in this case.

Now, if I use another color and draw the absorption spectrum of the accepter you see this is the absorption spectrum of the accepter. Now you see the photon which are emitted at this wavelength this wavelength this wavelength cannot be absorbed by this (Refer Time: 11:46) accepter right cannot be absorbed by this accepter one of the condition is the delta e has to be matched with the incoming photon h mu right that should match otherwise it is not possible. So, this is my absorption spectrum of the accepter. So, it is not possible right; however, if the absorption spectrum is not like this, but it is like this this is absorption spectrum of the accepter.

In this case what you can see that the photons which are emitted at these wavelengths starting from here to here, the absorption of this A is actually matching there is a certain probability that these photons will be absorbed. Obviously, the absorption cross section at the different wavelengths will be different. So, the probability of the different h mu will be different, but there is some probability there is a probability of the absorption.

So, what we cans tell that these common area; if this common area is large then the emitted photon has a good probability for the absorption by this accepter A. If this shaded region is small then the probability that the emitted photon will be absorbed by this accepter will be less and this shaded region is known as spectral overlap integral and denoted as j mu bar.

So, this j mu mu bar I mean just is a inverse of the wavelength alright and they say usually the unit we referred is as a centimeter inverse and the expression for this that should contempt the absorption spectrum, the emission spectrum, emission spectrum of the donor and the absorption spectrum of the accepter right and this is generally evaluated as integration 0 to infinity right florescence spectrum or emission spectrum of donor.

Obviously, this is a plotted as a function of wavenumber you know you can I already discussed that we can change the units right that it change from the wavelength to wavenumber to frequency whatever you want right. So, I am just (Refer Time: 14:49) using here the wavenumber into absorption spectrum absorption spectrum, and epsilon the more and less coefficient is same it is just divided by the concentration into path length. So, this is just a epsilon this is same thing epsilon of the accepter which is plotted against nu bar d nu bar. So, this is my overlap integral.

So, here this number three these three point is telling me if this overlap integral j mu bar is more than this is more favorable the energy transfer is more favorable. Like considering all this thing if you now try to find out the fraction let me write the fraction of emitted photon by D star right and consequently absorbed by A. So, that can be evaluated and that is generally expressed as a small a and then I should have all these things over here right in the expression I am not (Refer Time: 16:17) I am just writing the expression, but all these this one this one this one everything should be present in this formula alright.

So, this is this is equal to 1 over phi d 0 where this phi d 0 is the quantum yield of the donor in absence of I should write somewhere I will write it. So, 1 of our phi d is 0 C A this is concentration, and this is that path length right of the sample and then integration 0 to infinity F D nu bar epsilon a nu bar d nu bar where phi d 0 is fluorescence quantum yield of the donor in of absence of the accepter and C A is your molar concentration of A and A is the thickness of the sample.

Alright, now I will tell you the another possibility, that other possibility is that you have excited the donor, but before the donor actually emitted the photon the energy is transferred to the accepter A. So, in this case this is the radiative energy transfer as I told you and the other possibility is that non-radiative energy transfer and this radiative energy transfer is a one step process right.

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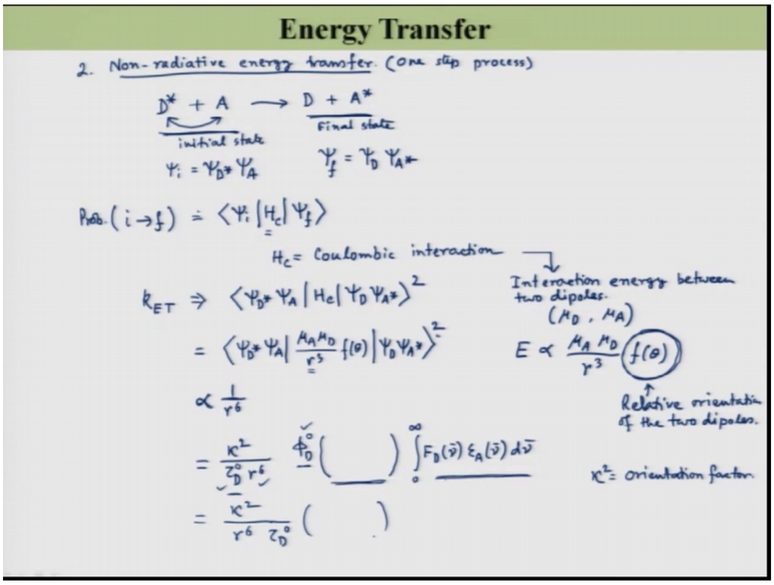

Energy Transfer

2. Non-radiative energy transfer. (One step process)

$$\underbrace{D^{*} + A}_{\text{initial state}} \longrightarrow \underbrace{D + A^{*}}_{\text{Final state}}$$

$\Psi_i = \Psi_{D^*} \Psi_A$ $\Psi_f = \Psi_D \Psi_{A^*}$

$$\text{Prob.}(i \to f) \doteq \langle \Psi_i | \underline{H_c} | \Psi_f \rangle$$

H_c = Coulombic interaction → Interaction energy between two dipoles. (μ_D, μ_A)

$$E \propto \frac{\mu_A \mu_D}{r^3} \boxed{f(\theta)}$$

↑ Relative orientation of the two dipoles.

$$k_{ET} \Rightarrow \langle \Psi_{D^*} \Psi_A | H_c | \Psi_D \Psi_{A^*} \rangle^2$$

$$= \langle \Psi_{D^*} \Psi_A | \frac{\mu_A \mu_D}{\underline{r^3}} f(\theta) | \Psi_D \Psi_{A^*} \rangle^2$$

$$\propto \frac{1}{r^6}$$

$$= \frac{\kappa^2}{\tau_D^0 r^6} \phi_D^0 (\underline{\quad}) \int_0^\infty \underline{F_D(\bar{\nu}) \varepsilon_A(\bar{\nu}) d\bar{\nu}}$$

κ^2 = orientation factor

$$= \frac{\kappa^2}{r^6 \tau_D^0} (\quad)$$

So, let me show you that. So, the other option is D star was there and in presence of A this d becomes D star become D and A become A star and this is fall under this category of non-radiative energy transfer which is a one step process. Now obviously, I have to discuss about that what are the variables on which these non-radiative energy transfer depends.

So, if you look at here; obviously, it depends on the lifetime of this donor because if the excited state will remain there for a longer time then only such kind of interaction is possible for example, if you just excite and immediately it will come back then there is no time for the interaction with this molecule. What kind of interaction? That is also important that I am coming after few minutes, but what you understand is that the rate of non-radiative process must be low so that the molecule will stay for longer at the excited state then only the interaction is more favorable alright if it is immediately comeback then interaction is not that favorable. So, it depends on the quantum yield because that is also depends on the radiative non-radiative path (Refer Time: 20:38) and it depends on the lifetime this is the same thing alright in absence of the accepter right it also.

Now, here it is not like that that photon is emitted right and that photon is absorbed by this A. So, the concentration that how many are present that is not important, the important parameter in this case is that because they have to interact these d and a these two have to interact with each other. So, the important parameter in these case is how close are they if they are far apart then obviously, (Refer Time: 21:18) any kind of interaction I (Refer Time: 21:20) I will discuss what type of interaction it is, but if the distance are far apart then those interactions are not visible as understandable if they are close by then; obviously, those interactions are visible right.

So, let me produce by telling you that (Refer Time: 21:43) both these donor and this accepter right they are dipoles right in rare case they are not dipoles right, I mean that they have some permanent dipole movement that same dipole movement I was talking about when we were discussing the (Refer Time: 22:00) on the fluorescence like a it has a dipole movement of mu D. Mu D in the excited state mu D in the ground state they may be same may be different, but in most of the cases they have this dipole. So, in this case the d has a dipole or D is a dipole and this A is also a dipole.

So, there is a possibility of dipole dipole interaction. If I now describe this situation this d star and a is as my initial state and D plus A star as my final state, this transformation form the initial to final must be accompanied with some (Refer Time: 22:50) and that is the interaction between this donor and the accepter right. So, if I write the wave function of this initial state as psi I which is psi D star psi A and the wave function of this final state is psi f I i am just writing as a product of this (Refer Time: 23:24) wave function of d and a. So, in this case this is psi d (Refer Time: 23:26) psi A star then I can write that particular transition right; that means, i to f right or I can write this probability of i to f. So, this is written as psi i that is my (Refer Time: 23:47) psi f.

Now, question is that what is this h? As I said that they are dipole and nobody stop us to tell that is a columbic type of interaction. So, if I take this h as a columbic type of interaction let me write that. So, here it is my H is columbic interaction and in this case I will simply write this as H c alright columbic type of interaction. So, this rate constant of this energy transfer k ET will be given by psi D star psi A H c psi D psi A star square right. So, this will be rate constant of this energy transfer energy transfer.

Now, the interaction energy between the 2 dipole right now let me talk about this this columbic interaction. So, the interaction energy between two dipoles, here I said that one is mu d another is mu A, one is for the donor another is for the accepter. So, that is proportional to mu A mu D by r cube and one factor if theta which actually tells me about the dilutive orientation of this two dipole.

So, it will such kind of form; that means, this k ET that rate of rate constant of this energy transfer right I should write as psi D star psi A mu A mu D by r cube f theta psi d psi A star. So, here you see this r cube term is over here and here is a square term. So, whatever it is whatever the expression is I what I will get is this is proportional to r to the power 6, whatever the other term is present over here I will get that this k ET is proportional to 1 over r to the power 6. That means, here you can see that the distance between the donor and accepter is really one of the important parameter to decide about this fate of the energy transfer from D star to A to D to A star right

We will going to see obviously the full expression and as I told you this also should depends on this f theta that is also important that is the relative orientation of the two dipoles right.

Now, if I write the complete expression right it will look something like this this kappa square by I will define this term tau D to r to the power 6 phi D. And then there is some constant which I am not writing over here and then integration 0 to infinity if t mu bar f epsilon A mu bar D mu bar. You see as I have commented it depends on phi D, it depends on tau D it depends on the distance, it depends on this overlap integral; obviously, it has to it depends on the overlap integral because the energy should match otherwise this is not possible. So, it depends on the overlap integral and it also depends on this factor kappa square where this kappa square known as the orientation factor orientation factor.

Now, considering that; obviously, this tau D I should write as tau D 0 because this is the lifetime of the donor in absence of the accepter right and obviously, I should also write this phi D as phi D 0 because this is a fluorescence (Refer Time: 29:42) of the donor in the absence of the accepter. So, these whole equation right can be converted in a small equation if you consider the fact that here this is a constant term this tau D 0, phi D 0 is a constant, because it is a property of the donor this overlap integral is a constant for a particular pair, if I define this is my D this is my A then overlap integral has to be constant for a particular solvent.

So, all these things are constant right only this kappa square is not constant because it depends on the relative orientation of the donor is donor is like this accepter is like this kappa is defined donor and accepter are like that kappa will is different like this, so this kappa square. So, I can convert this equation as kappa square, and obviously r is not a constant because the distance between donor accepter can change can vary by r to the power 6, I deliberately also take this tau D 0 over here right and rest of the things are constant.

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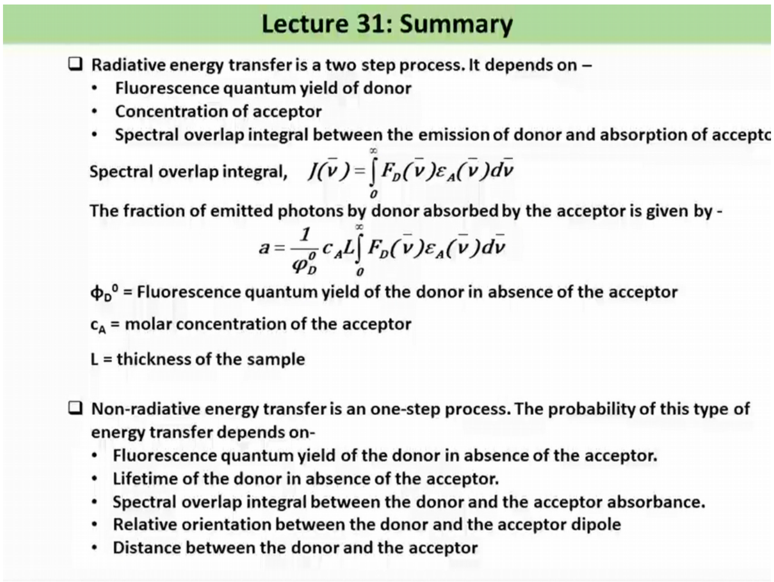

Lecture 31: Summary

- ☐ Radiative energy transfer is a two step process. It depends on –
  - Fluorescence quantum yield of donor
  - Concentration of acceptor
  - Spectral overlap integral between the emission of donor and absorption of acceptc

Spectral overlap integral, $J(\bar{\nu}) = \int_0^\infty F_D(\bar{\nu})\varepsilon_A(\bar{\nu})d\bar{\nu}$

The fraction of emitted photons by donor absorbed by the acceptor is given by -

$$a = \frac{1}{\varphi_D^0} c_A L \int_0^\infty F_D(\bar{\nu})\varepsilon_A(\bar{\nu})d\bar{\nu}$$

ϕ_D^0 = Fluorescence quantum yield of the donor in absence of the acceptor

c_A = molar concentration of the acceptor

L = thickness of the sample

- ☐ Non-radiative energy transfer is an one-step process. The probability of this type of energy transfer depends on-
  - Fluorescence quantum yield of the donor in absence of the acceptor.
  - Lifetime of the donor in absence of the acceptor.
  - Spectral overlap integral between the donor and the acceptor absorbance.
  - Relative orientation between the donor and the acceptor dipole
  - Distance between the donor and the acceptor

Let me stop here, and we will continue our discussion on next day.

Thank you very much.