Basics of Fluorescence Spectroscopy
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Lecture – 22

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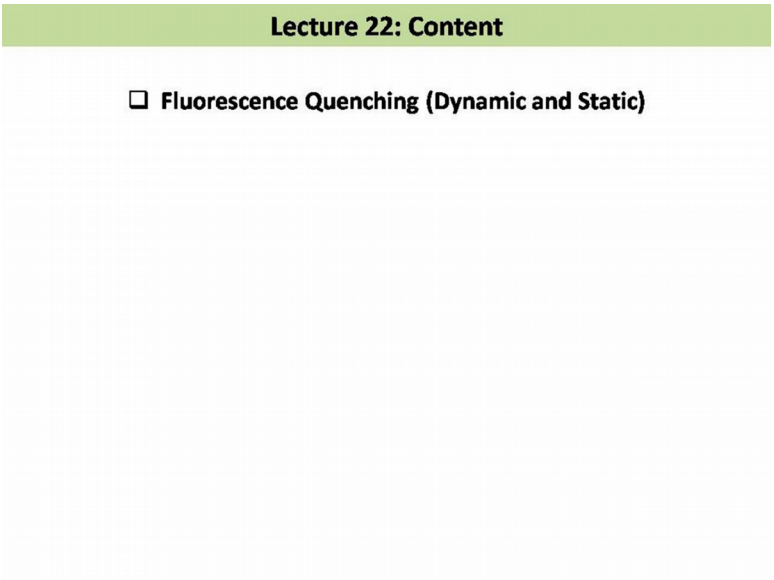

Welcome to the lecture number 22. Till last lecture what we have finished is the instrumentation for the lifetime measurement, and today I would like to start a new topic that is the effect of intermolecular processes on fluorescence.

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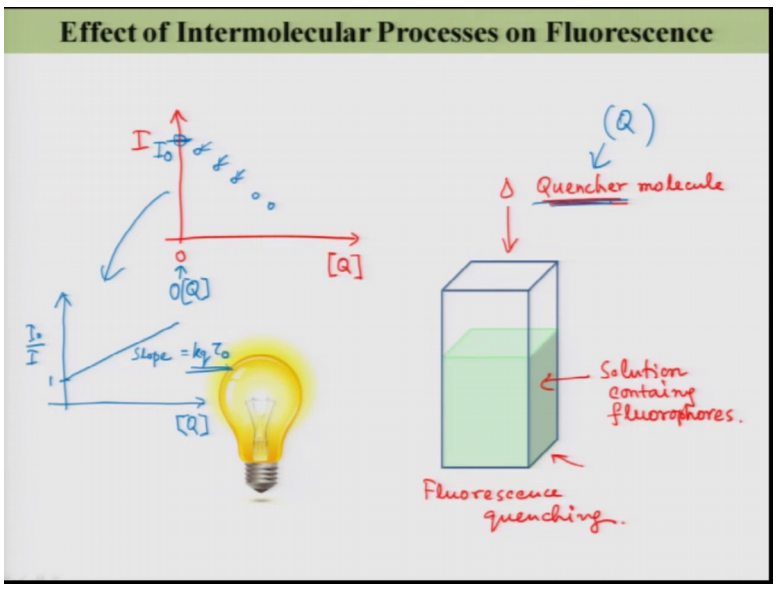

And basically what we will going to discuss is that how the fluorescence intensity will change because of different intermolecular processes at the excited state of the species. So, the most well known or well discussed phenomena for such kind of process are broadly represented as the fluorescence quenching. And in the fluorescence quenching, let us say you have this fluorophore solution in your cue head and upon excitation of suitable life source at the molecule will start fluorescing. So, let us say this particular molecule is giving bright green color fluorescence over here.

Now, on addition of some outside agent as I am adding over here we will see that just I am adding one drop of the other molecule in it and the color is likely not that strong fluorescence right, the fluorescence intensity decrease. Again if I add little more of this compound from the outside then the fluorescence intensity decreases again. And that will keep on go on as long as we see that concentration dependent fluorescence intensity change of this species. So, this phenomenon is known as fluorescence quenching.

So, in this case what we will going to see is if I plot the fluorescence intensity as a function of concentration of that other species right whatever I was adding over here. So, if I plot the fluorescence intensity as a function of the concentration of this compound which I am adding generally known as the quencher molecule. So, I was adding quencher molecule to my solution containing fluorophores.

So, from here what I can do I can simply plot the fluorescence intensity as a function of concentration of this quencher over here. So, let me do that. In that y axis let me plot fluorescence intensity just simply denoted by I and then x axis is the concentration of quencher. So, if the fluorescence spectrum is very strong right. So, intensity is very strong when there is no quencher; that means, 0 quencher. So, I will start from this point, I will start from this point, and then as I will add this quencher as s the concentration of the quencher will increase in this solution then the fluorescence intensity will keep on decrease.

So, it will start from a value so that depends on the excitation light source the concentration of the fluorophore present in the solution like these are the main thing. And obviously, the quantum yield of the fluorophore. So, depending on that I will get particular value of intensity. And then that intensity value will keep on decrease like this way as I will change the concentration of the quencher.

However, in generally this type of representation was not done in fluorescence quenching experiments rather what people do is just plot I 0 by I versus concentration of the that molecule quencher molecule. This quencher is I just represented as Q. So, if I now plot I 0 by I what is that I 0; I 0 is the intensity 0 means for 0 Q then that is called the I 0. And then all those others are I, I, I, for a different value of Q. So, what I will going to get? When Q equal to 0 then I 0 by I is equal to I 0 by I 0 this is equal to 1. So, it will start from the value 1. As I am going to increase the concentration of the quencher the fluorescence intensity will decrease. So, the I 0 by I this value will be greater than 1. So, that total quantity will be greater than 1. So, what you will going to get? You will get such kind of plot.

And with the specific slope and that slope is equal to k q tau 0. I will come with this derivation later on. So, this kind of value that whatever I have written in this slope is called the strum homer quenching constant which has some significance which we can discuss later on, but this is the whole as a phenomena of the fluorescence quenching. So, what is going on I am going to add some molecules which is referred as the quencher; quencher means it is quench something that is the fluorescence.

So, by addition of those outside agents, outside molecule to a solution of fluorophore which is actually giving fluorescence emission from the sample, the fluorescence intensity is quenched. So, fluorescence intensity is becoming lower and lower as we add those type of molecule like the quencher molecules to the solution. And that is best represented in the literature or in several books like I 0 by I. If you want you can also represent it by just simply plotting I versus Q, but generally it is done for the continues I 0 by I versus Q where you will get straight line and the slope is known as the strum homer quenching constant. I will come to the derivation of this as I said.

Now, let me give you some example; example of the reason for this fluorescence quenching. Now obviously, we will going to ask that why such fluorescence intensity is decreasing by the addition of the quencher.

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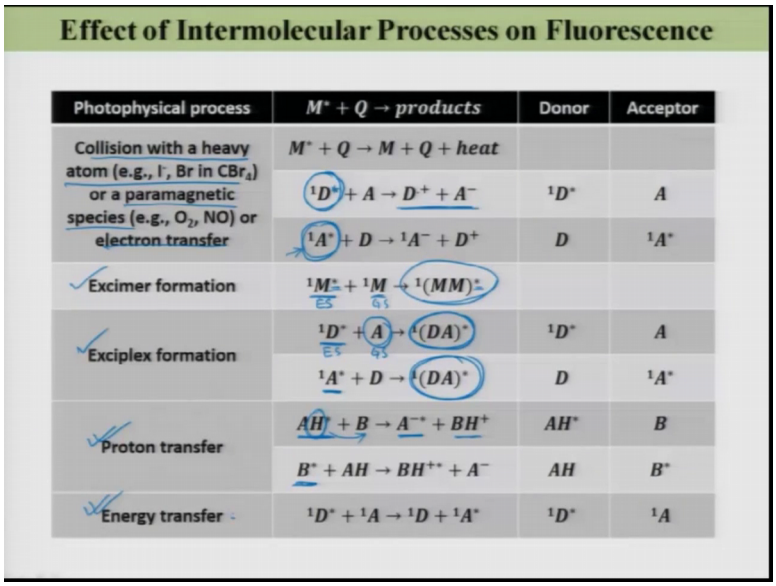

There could be several reasons: first I have written the collision with heavy atom, that could be one of the reason or collision with parametric species or electron transfer. So, in this case let us say for the electron transfer the fluorophore which you have is actually in the excite state. And you remember that excited state is denoted by putting A star marks after the molecule. So, here I put the molecule as D and here you see A star mark; that means that this molecule D which is known as the donor is in the excited state.

Now if some other species that Q I was talking about that Q here is A, if A comes and interact with the D star. So, that this D star is getting deactivated it somewhere it constant. So, you see here there is no start that means this is a not in the excited states; that means, fluorescence will not come from this species. Eventually you can also excite the A star like a the acceptor in the excited state and this is now your fluorophore. Your fluorophore not necessarily be the donor it also could be the acceptor. So, this for the electron transfer, but probably will not going to discuss this electron transfer process because of this basic nature of this course. But I just for your information that what are the different process which are responsible for this fluorescence quenching. We will discuss fluorescence quenching in detail, but those processes right that is what I am discussing here maybe you will not going to be covered in this course.

So, the other type of mechanism is excimer formation, like you see here this molecule M is in the excited state, this star look at this star marks. And it may interact with another

molecule which is not in the excite state and giving something like this where the fluorescence of these guy is totally different than the fluorescence of these guy. It means that you will going to see the decrease of the fluorescence intensity for this M star molecule. It also could be the exciplex formation.

In this case these type of molecule is interacting with the different type, here this is M, this is M, this is in the excited state this is in the ground state and this is in the excited state, but here is totally different molecule. Although this is in the excited state this is also in the ground state, but they are different molecule. And they can also form such kind of complex over the fluorescence signal is quite different than that of A star or D star and you will see the decreasing the emission intensity of D star or A star. So, this is nothing but another type of fluorescence quenching.

We can also have this photon transferred over here. So, probably this one I will be going to discuss little bit. In this case that if the molecule which you are going to use as a fluorophore that means, which will going to give you the emission under photo excitation; that is my fluorophore; that is my fluorescence. In that case this one of the hydrogen may be transferred to one base to make the conjugate acid and the conjugate base of this molecule.

So, in this case the nature of emission of A H star and A minus star they are quite different. So, you will going to see the number of A H star is being reduced because of this proton transfer and the formation of A minus star is taking place. So, as A number of A H star molecule is decreasing in the solution so obviously, the fluorescence intensity will decrease which is nothing but a kind of quenching. There is a fluorescence intensity will decrease. That means, the fluorescence in fluorescence will quench for A H star emission.

Similarly, you can I can talk about this B star. So, this is the exactly the same thing and you can also have this energy transfer as a responsible for the fluorescence quenching of a particular band. This one will going to discuss in detail later on, this is a interesting topic.

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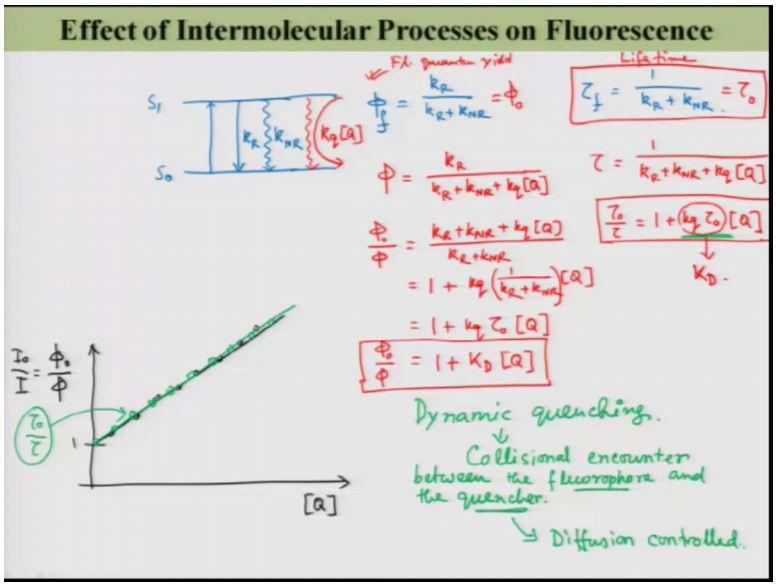

So, let me draw one diagram over here. This is again very simplified Jablonski diagram, as I already discussed. So, let me draw the energy level of a molecule under investigation which is my fluorophore. So, here is my S 0 state and here is my S 1 state. So, if the transition is allowed transition under suitable excitation I can promote molecules from S 0 state to S 1 state, that we have already seen.

And after being excited; so the fate of excitation we will already discussing that molecule will come back from the excited state to the ground state mainly by two different pathway: one is designated as the radioactive pathway, where the dissipation of the excess energy is in terms of light; and the another types of pathway is the non-radioactive pathway, where the dissipation of the excess energy is in terms of heat you remember.

So, this is my radioactive pathway k R and I would also have this non relative pathway k NR. And I have already defined this fluorescence quantum yield of this molecule as k R by k R plus k NR and the lifetime of this I have already defined as 1 over k R plus k NR. Now what I am telling you that obviously, as you have seen that on addition of this Q is actually decreasing its fluorescence intensity and that decrease of the fluorescence intensity it depends on the concentration of this quencher molecule.

So, there must be another pathway by which there is a deactivation and this deactivation is type of non-radioactive deactivation, because in this case there is no emission; if there

is emission of photon then the fluorescence intensity will not going to be decrease. So, here it is fluorescence intensity is decreasing; that means, this is a kind of a non-radioactive process. And it depends on the quencher concentration. So, I write this as k q into Q.

So, this is another pathway by which the molecules are coming back from the excited state to the ground state. So, as these are in absence of that molecule; these are means, these expression of the quantum yield right fluorescence, quantum, yield and the expression for the lifetime, as their in absence of this quencher molecule I would rather replace this term as phi 0 and tau 0. Now in presence of this quencher another pathway is present k q into Q. So, in that case what will happen? If I want to write this phi then this phi I will write as k R, so this is only the radioactive process by which the photon is emitted and all others or whatever this extra k q into Q this is the non-radioactive process.

So, I write here k R plus k NR plus k q into Q, and in this case I will also write like that way 1 over k R plus k NR plus k q into Q. Now what I will do, I will take the ratio of phi 0 by phi; is not it. So, if I take this ratio phi 0 by phi what I will get is k R plus k NR plus k q into Q divided by k R plus k NR. So, this is equal to 1 plus k q into 1 by k R plus k NR into Q so this is equal to 1 plus k q, what is this? This is nothing but tau 0 see here tau 0 I have defined in this case tau 0 into Q. And this is this k q into tau 0 is written as K D Q or somebody also write as k s v and this is called the stand homer quenching constant.

If you do the same thing over here like if you take tau 0 by tau then what you will get; you will get exactly the same expression, because here this k R and k R cancels so this is nothing but that the same expression. So, in this case you will also get 1 plus k q tau 0 into Q. So, what you have seen? This phi 0 by phi is having such kind of equation; tau 0 by tau is also having such kind of equation, where this quantity is k D strum homer quenching constant.

So, as I told you that if you plot now phi 0 by phi: plotting phi 0 by phi and I 0 by I is same. Why so, because same under the constant excitation light intensity. So, this is same as I 0 by I that what I was telling you just few minutes back. So, if I plot, this plot will start from 1 and you will get this type of different values as a function of Q.

Now, if you plot tau 0 by tau instead of phi 0 by phi see here; the slope is exactly same. So, you should get exactly same slop as phi 0 by phi- tau 0 by tau and phi 0 by phi are exactly same. In this case just try to visualize this thing there is a fluorophore which is giving fluorescence, it has its own radioactive pathway, own non-radioactive pathway. Now its external agent which is called the quencher is coming interacting with the fluorophore, because of the collision. So, it is interacting and then it is creating a further non-radioactive pathway which I have denoted as a k q which is also depends on the concentrations. So, k q into Q I have written in the term. And then the fluorophore is getting quenched right because of the creation of the extra non-radioactive pathway in its photon physics, clear.

Now question is that, what will happen if the viscosity of the medium will increase? If the viscosity of the medium will increase then those quencher molecule will not be able to interact with the fluorophore in that way. That means, the quenching phenomena will not be that strong. In other words these whole phenomena can be represented as that this is kind of a dynamic process, like this is a collision between the two molecule is responsible for such kind of quenching. The quencher molecule should come and interact with the fluorophore then only such kind of process will takes place. So, this is a dynamic process.

So, this type of quenching is known as dynamic quenching. So, this is resulted from the Collisional encounter that is what I said right now right between the fluorophore and the quencher. So, the fluorophore and quencher interaction like this fluorophore and quencher interaction should be text should takes place right within the lifetime of the property this is also an interesting thing. So, this is also called as the collisional quenching and please note that this is Diffusion controlled.

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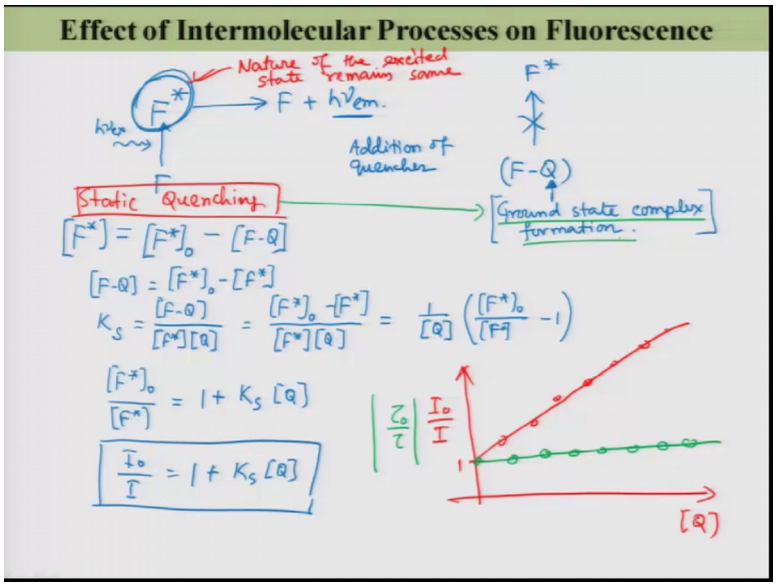

Now, let me discuss another type of phenomena: suppose you have a molecule F which is in the excited state will give you light, so F plus h nu emission. And you have started with some quantity of F and when you excite it you will create this F star and then F star will form the F, because of it has a radioactive process it also has a non-radioactive process so depending on that you will get this h nu emission.

Now what if when you are adding the quencher here? So addition of quencher will take away some fraction of the F and complex with it so that you are unable to excite that fraction of F from the ground state to the excited state. What I wanted to say is that, now some F Q complex which you cannot excite to the F star state. That means, the concentration of the F star state will decrease if the concentration of the F star state will decrease that means, the fluorescence intensity will decrease. If you increase the quencher concentration Q then more and more F will get complex with Q. So, this is the ground state phenomena. So, here is the ground state complex formation in this case, is not it.

Now that I can also equate it; let us designate the fluorescence intensity as is proportional to the concentration. So, I can write the fluorescence intensity is proportional to the concentration. So, let us say the fluorescence intensity is F star 0 when there was no quencher add it. That means what about the F star you can create from the ground state by your suitable excitation source that is the concentration which is obviously

proportional to the fluorescence intensity of this sample. So, I can just simply take F star 0 is my initial fluorescence intensity without addition of the quencher molecule.

Now once you will add this quencher. So, fluorescence intensity will be different than this, so let us write that F star is the fluorescence intensity in presence of quencher, then I can write F star equal to F star 0 minus something minus F Q. So, some fraction of the fluorophore is getting complex with Q which I cannot excite anymore. And all the remaining F are excited to the excited state. Or I can write F Q, that concentration of F Q equal to F star 0 minus F star.

So, I can write some equilibrium constant F Q divided by F star into Q, so this is equal to F star 0 minus F star divided by F star into Q, this is equal to 1 over Q into F star 0 by F star minus 1. So, rearranging F star 0 by F star is equal to 1 plus K s into Q. And this F star 0 by F star is nothing but I 0 by I that is what I would like. So, I can write I 0 by I is equal to 1 plus K s into Q.

You see this form of this equation, and you look at the form of the other equation you see this form phi 0 by phi; this form phi 0 by phi equal to 1 plus k D into Q, here I 0 by is equal to 1 plus K s into Q, they are exactly same. So, in this case also write this K s means where the ground state complex formation takes place and because of that the fluorescence intensity is decreasing as I am adding the quencher molecule in my medium.

So, in this case the mechanism is completely different, in the other case the mechanism was the quencher molecule is coming and interacting with the excited state of the species which is responsible for the fluorescence, and it is creating another pathway other non-radioactive channel so that the fluorescence intensity v of the molecule is decreasing. Because fluorescence intensity means quantum will that means, k R by k R plus all the non-radioactive processes.

But in this case there is a ground state complex formation is taking place. As this ground state complex formation is taking place I am not going to have enough fluorophore in the excited state, and that is why the fluorescence intensity is decreasing. The two phenomena are completely different, the mechanism is completely different, but the equations are very similar or exactly same.

So, in this case also and so type of quenching is known as static quenching; I will write here somewhere, so static quenching. So, in this case also if you plot the fluorescence intensity or the ratio I 0 by I versus concentration of Q what you will going to see you will also going to see a straight line with intercept 1; exactly same.

Now question is how will going to distinguish whether it is a static quenching or it is a dynamic quenching. Here you see I am exciting the molecule in the excited state, the nature of the excited state remain same only some part of the molecules cannot be excited to the excited state because of the ground state complex formation. The nature of the excited state remains same; same to what before addition of the quencher; same two before addition of the quencher.

So, there is no change in the excite state property; that means, whatever the value of k R and whatever the other values of k NR they remains same. That means, there will be no changes in the lifetime please note that. When I will going to plot tau 0 by tau in similar fashion what you will going to see always the ratio is one even you increase the quencher concentration, that is because lifetime does not depend on the concentration of the species or lifetime is the property of the excited state.

And in case of static quenching the ground state complex formation is taking place which is just removing a fraction of the fluorophore from my observation. So, excited state remains same. So, I can readily distinguish whether it is static quenching or dynamic quenching by measuring intensity as a function of quenching as well as my measuring the lifetime as a function of quencher.

So, in this case is see the measurement of lifetime is so important only using this lifetime data you will be able to tell whether this quenching is static quenching or is a dynamic quenching. Let us finish here, and we will continue our discussion on the next lecture.

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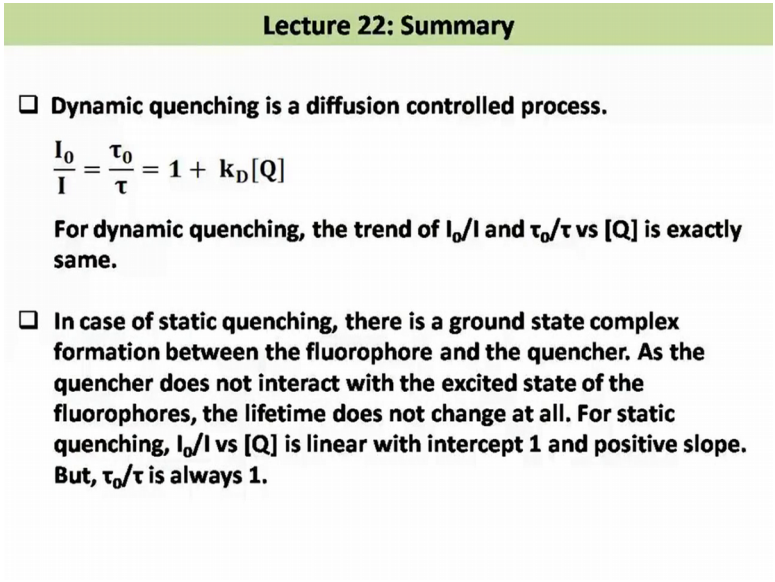

Thank you very much.