Biochemical Engineering

Prof. Dr. Rintu Banerjee
Department of Agricultural and Food Engineering
Asst. Prof. Dr. Saikat Chakraborty
Department of Chemical Engineering
Indian Institute of Technology, Kharagpur

Module No. # 01
Lecture No. # 32

Effects of Ligand Depletion and Multiple Receptors on Binding Kinetics (Contd.)

Welcome back to this lecture on Biochemical Engineering. If you remember what we did in the last class, was essentially we are looking at multiple receptors. And, what happens when the, in the species, in the complex, in the ligand binds to multiple receptors. We talked about the fact that these multiple receptors essentially bind independently to the ligand and the binding of one, does not influence other. And then, we said, is it possible to determine separately, whether these multiple receptors receptor 1 bound the amounts that receptor 1 binds to the ligand, the amount that receptor 2 binds to the ligand. We said, well, it is not always easy to figure out because what we do is, we label the ligands essentially.

So, the ligand, if there is a one kind of ligand, it will either bind to receptor 1 or bind to receptor 2. And, there is, you know we are just looking at the fluorescence or the some kind of labeling. And, it is not possible to figure out in general, whether receptor 1 binds or it is binds receptor 1 or it binds to receptor 2. In some extreme cases, obviously it is possible. And, what are those cases? So, the case that we talked about was, when a receptor of a particular kind has a much higher binding infinity than the receptor of the other kind.

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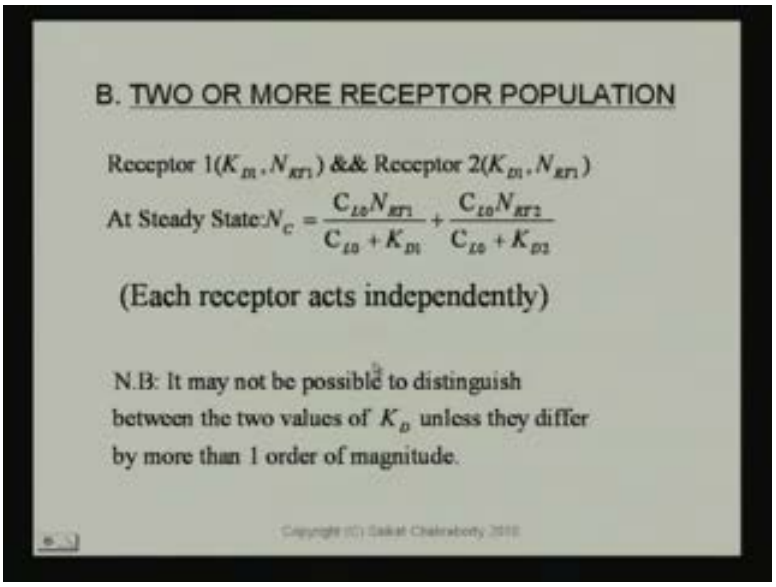

And, If you remember, so we, this was what we look doing, if I will, if I go to the screen now. So, a receptor 1 and receptor 2 bind. And, this steady state concentration of the complex is the amount of receptor 1 is binding to the ligand plus the amount of receptor 2 is binding to the ligand. So, the first term you find here which is N C, C L naught N R T 1 over C L naught plus K D 1; that is the amount of receptor ligands bind to receptor 1. And, the second term that you see here, C L naught N R T 2 over C L naught plus K D 2; that is the receptor amount of ligands binding to receptor 2. So, the fact is that, each of this receptors act independently and bind independently and there is no influence of the binding of 1 receptor to the binding of the other. But, it is possible as I said to distinguish between the two values, if the K D, that is the dissociation rate constant of receptor of the biding of receptor 1 is much much higher or much much lower than the receptor binding rate constants of the second one.

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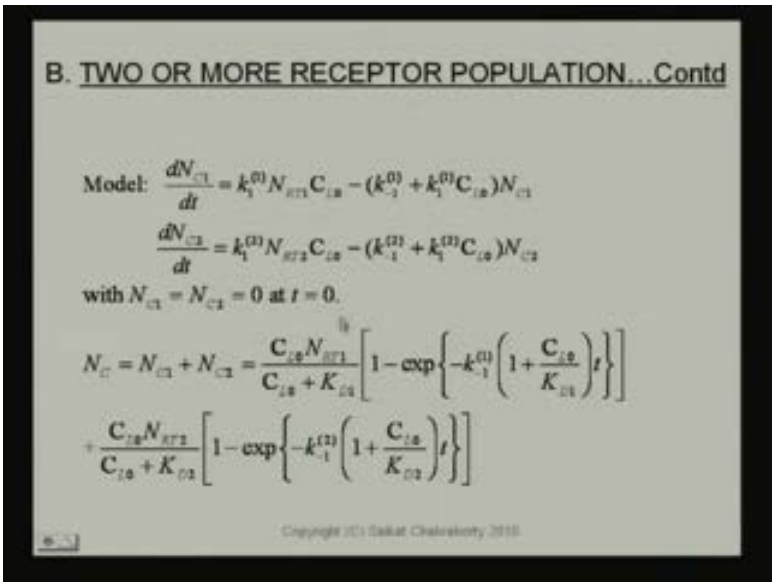

Now, if you remember, so, we would went through the calculations and I am not going to repeat all of that. And, when we did the calculations, we took the case when the amount of complex that was there at the beginning was zero and we did all the calculations.

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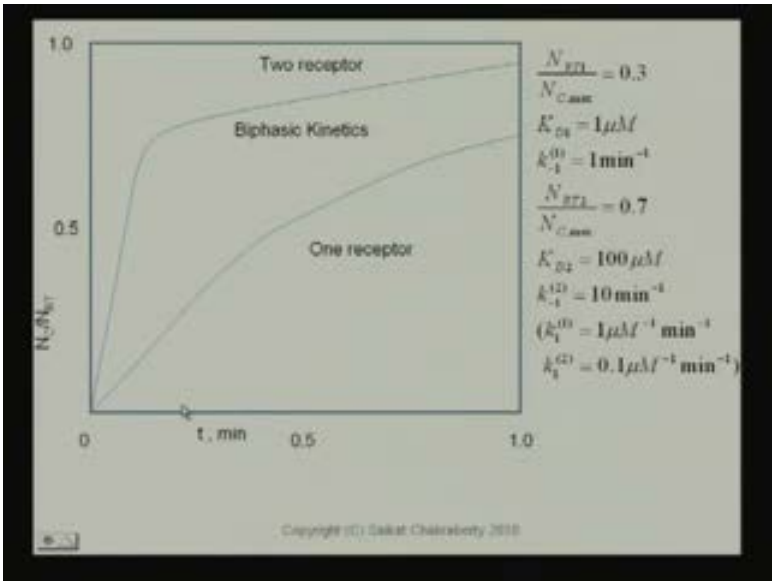

And, what I want to attract your attention to is this factor this biphasic nature of the

curve. So, what you see over here is N C over N R T and this is time. So, you have a single receptor and the case of a single receptor, the curve is monophasic; whereas in the case of two receptors, the curve is biphasic.

Now, as you can see over here, these are data here and I am going to look at that. So, look at the K D 1. The K D 1 is 1 micro molar and the K D 2 is 100 micro molar. So, there is the two orders of magnitude difference between the dissociation rate constants of 1 and 2.

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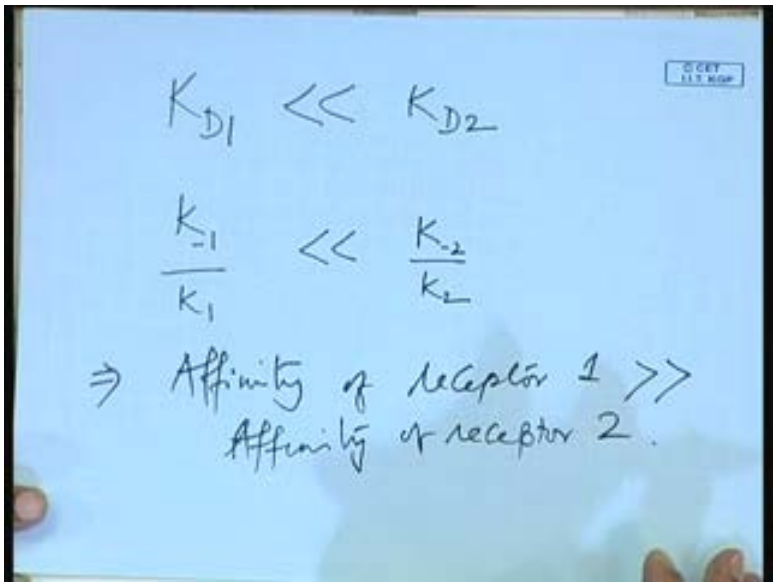

So, if K D 1 is hundred times, so, here if K D 1 is much much smaller than K D 2 as the case is, then K 1 K minus 1, let us say over K 1, that is the backward rate constant over the forward rate constant is much smaller than K minus 2 over K D 2; which means, affinity of receptor 1 is much much higher than affinity of receptor 2. So, this is what we concluded; that the affinity of receptor 1 is much much higher than receptor 2, if this is the case.

And, these are cases, when we can really distinguish between the two kinds of receptors because as I just showed in the biphasic response that I showed that we had. So, what does this lead to, if K D 1 is much much smaller than K D 2? What this leads to is that in

the exponential graph that you have, the slopes of the exponential graph all points of time will be very different. And, what you see is that, there is clear biphasic nature; or in other words, the slope increase in a certain way. And, then they decrease suddenly, say if I can go back to that screen quickly, see, you see that this is the slope, say some kind of an exponential. But, this exponential slope of this curve, whether it is exponential or something close to linear does not matter; is very very different from the slope of the next curve.

So, which means what? That, if these two receptors are there and the K D 1 of 1 is much much smaller than the K D of the other; or in other words, receptor 1 has a much higher affinity to than receptor 2. Then, this implies that receptor whenever you let these ligands loose on the system, so, whenever you let these ligands loose on the system, so receptor 1 will capture these ligands, so that ligands are around. So, receptor 1 will immediately come and bind with the ligand. So, as the result, the initial amount of binding that will occur will essentially be because that of receptor 1.

So, once we understand than then we realize that, yes the two slopes are very different from each other. So, initially you have a much higher slope and then the slopes decrease with time. So, what this implies is that for the case, when the slope is higher or in the initial time period, so as soon as you let the ligands loose on the receptors, the receptor 1 is binding with the ligand. So, this will essentially imply that the receptor, the slope first slope that is the higher slope that corresponds to the binding of receptor 1 to the ligand. It is not that receptor 2 does not bind at all, but the fact of the matter is that much of the binding that occurs, maximum most of the binding that occurs is because of receptor 1. And, if you come to think of it, you know, if you come to think of these two as comparative binding that is receptor 1 is binding comparatively to receptor 2, then what you realize is that because the rate constant or the affinity of receptor 1 is hundred times that of receptor 2. So, the binding should always be in the ratio, more or less on the ratio 1 is to 100 or 1 is to 99. So, if hundred ligands bind, 1 percentage, only 1 percentage of them is binding to receptor 2 and 99 percentage of them is binding to receptor 1.

So, how does this help us? How does this knowledge help us? Now, I gave you a problem at the end of the last class and I will go back to that problem. This helps us in

identifying the rate constant of the system because just as I said that when we label the ligand, there is no way for us to label the receptors, we are only labeling the ligand. So, when we label the ligands what happens is that, we can only figure out that what is the total amount of complex that has been found. And, there is no way for us to separate out the receptor 1 from receptor 2 or the constant rate constants of receptor 1 from receptor 2. But, what we essentially want to measure are the rate constants of receptor 1 and receptor 2. So, how do we go about it?

So, what we do? We choose the ligand, we purposely choose the ligand. So, think of that you are doing an experiment and how do you go about it. So, you can only label your ligands; you cannot label your receptors. So, what do you do? You purposely choose a ligand such that, it binds both to receptor 1 and receptor 2. But, the binding rate constant or the K D at the dissociation rate constant, the binding affinity of the ligand for receptor 1 is much much higher than the binding affinity for the ligand, for of the ligand for receptor 2. So, that is the highlight. That is the point that you need to exploit.

So, once you have understood that what you do is, you essentially figure out. So, if 1 is hundred times, the other and if you let the reaction happen for a certain period of time, initially what will happen is, if the receptor 1's affinity is higher, much much higher than receptor 2 what will happen is, all of it will go. The ligands will, almost of the ligands will actually go and bind with receptor 1. So, that is essentially that is going to happen. Then, what happens? As time progresses, receptor 1 are going to be more or less saturated. And, then is, when you see that bend in that curve that, just that I showed you just now.

So, receptor 1 is more or less saturated. As a result, what happens is, now receptor 2 starts to bind to the ligand. And, this is the mechanism or the hypothesis…, say that is that is what we are going to explain as we try and solve the problems.

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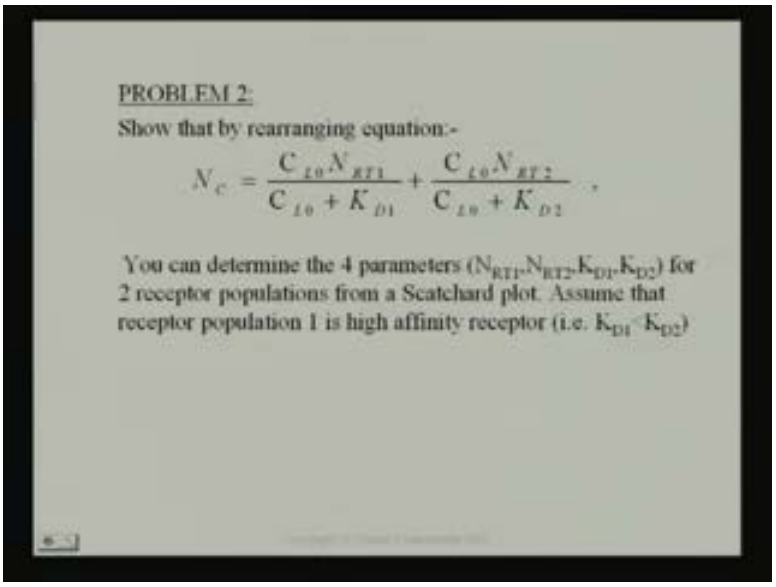

So, let us go to the problem. So, this is the problem that I gave you. And, what I asked you to do is that N C equals C L naught N R T 1 over C L naught plus K D 1 plus C L naught N R T 2 over C L naught plus K D 2.

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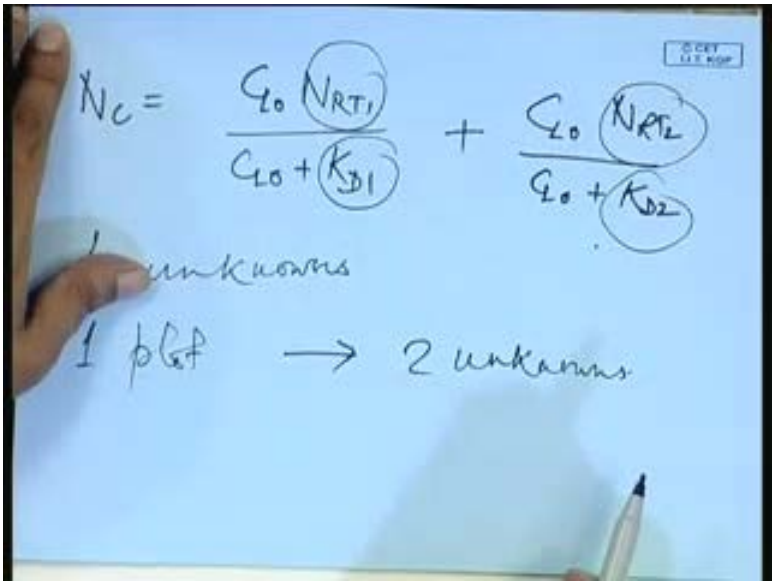

So, let us write this. So, and C equals C L naught N R T 1 over C L naught plus K D 1

plus C L naught N R T 2 C L naught over K D 2. So, one of the ways of tackling this is essentially, just what I said that, you know there it is very hard to be able to figure out. So, you have, just have a one curve, come to the think of it you just have a one curve. So, when you had… let us go back to this. And, when you had a single receptor, this was your scatchard plot.

So, when you had a single receptor, you had two unknowns; one was N R T and the other one was K D. And, you could draw a scatchard plot, which was linear. And, from the slope, you figured out N R T. K D, sorry. And, from the intercept, you could figure out N R T. So, two rate constants, two constants you needed to figure out. You had one plot. And, from the slope, you figured one and for an intercept you figured another. But, here, what happens is the the problem that I gave you. Sorry.

So, the problem that I gave you here, the problem with this is that you have one, two, three, and four. So, four unknowns and1 plot. So, how do you go? So, this can utmost give you two unknowns. So, how do you figure out the other two unknowns?

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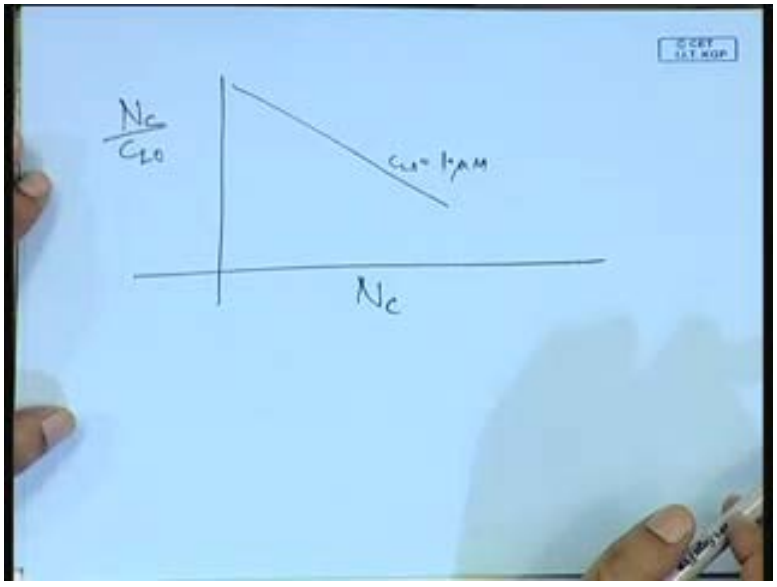

So, what we do is, we obtain a scatchard plot. Now, typically a scatchard plot would be linear. So, if you do N C over C L naught, over N C. Then for a particular value C L

naught, C L naught equal to say 1 micro molar or10 micro molar, something like that. This is what you get. This is your plot. But, in this case what will happen is that, given that K D 1 is much much lesser than K D 2.

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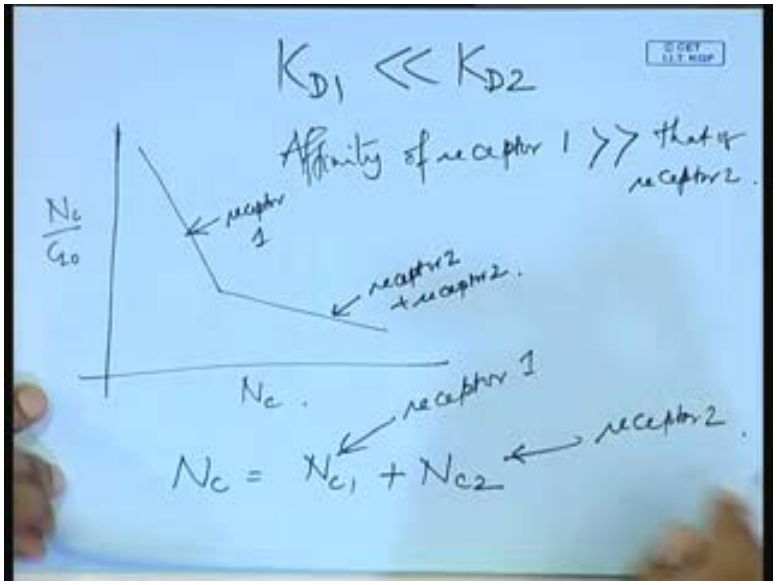

So, your plot is going to be something like, let us keep this formula next to each other. So, your plot is going to be something like this, and this. Why is that going to be the case? The reason is that N C equals N C 1 plus N C 2. That is what my total. So, it is complex form from receptor 1 and the complex form from receptor 2. That is what is going to be.

So, when that is the case, then when you have a limited amount of, a small amount of complex that is formed, what will happen is, much of it will be from receptor 1. Is it clear. Why is that going to be from receptor 1 because receptor 1's affinity or affinity of receptor 1 is much much greater than that of receptor 2. So, that is what will happen. So, you will have, most of it will be formed from receptor 1. Now, once receptor 1 is more or less saturated, most of the receptors are bound to the ligand, then only receptor 2 starts to form. So, then what happens is, you can exploit this as receptor 1. You can call this as almost as receptor 1. And, this would be primarily receptor 2 plus, may be receptor 1 also.

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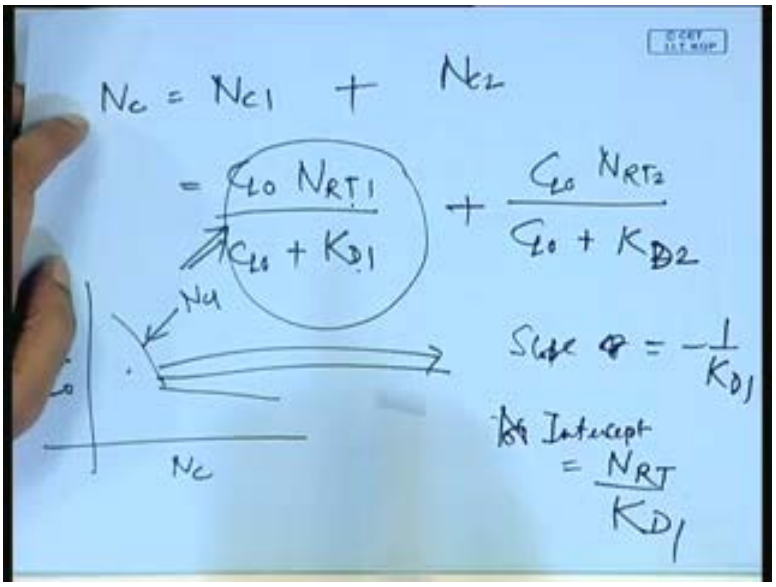

So, what we do now? So, we have our equation N C equals N C 1 plus N C 2, which equals C L naught N R T 1 over C L naught plus K D 1 plus C L naught N R T 2 over C L naught plus K D 2.

Now, for the first part of the plot, so, if my scatchard plot is something like this, N C over C L naught over N C, this N C that you get over here is essentially N C 1; this value. So, I can straight away from the. So, if I take this this part of the plot over here, I can straight away say that the slope of this equals the slope of the first part equals minus 1 over K D 1 and intercept equals N R T over K D 1. I can straight away say this. So, what happens is, essentially I use the first part of the slope to evaluate both my K D 1 and N R T 1. And, the second part I can calculate, I can assume that both receptor 1 and receptor 2 are present and I can go ahead and calculate everything I want. Or, second part you, I can make an even more simplifying assumption and say that this is equivalent to N C 2. In which case, the slope of this will give me minus K D1 over minus 1 over minus K D 2 and the intercept will give N R T 2 over K D 2.

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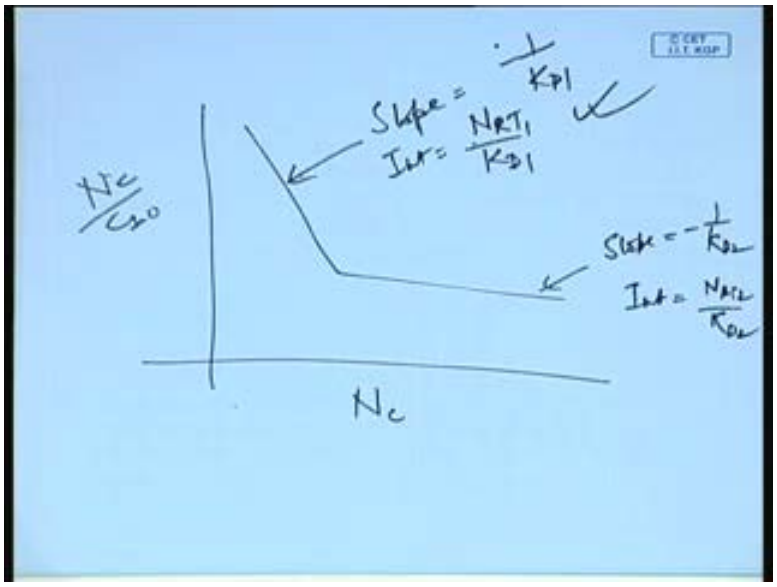

So, that is the possibility. So, I can do something like this. So, if that is the case, then slope, this is N C over C L naught over N C. Then, slope equals minus 1 over K D 1 intercept equals N R T 1 over K D 1 and slope here, can be minus 1 over K D 2 and intercept equals N R T 2 over K D 2. That is the possibility, or here you can assume both in receptor 1 and receptor 2 to be acting; in which case, you have to do a little more complicated calculation. But, this is something you can definitely take. So, that is a, that is the way we solve this. Otherwise, there is no other way of looking at it.

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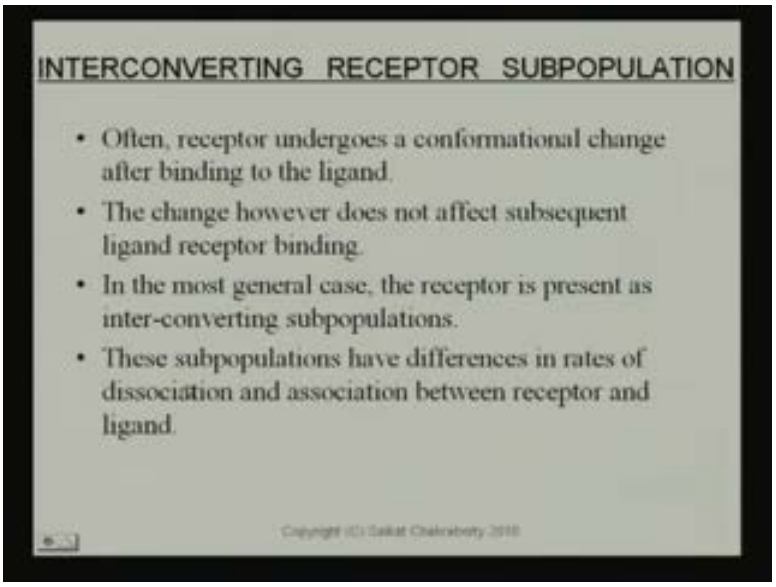

Now, let us look at some other things here, some other variations of the problem over here is, you can see the… what we are talking over here is, now, so we start to talk about here is interconverting receptor subpopulation.

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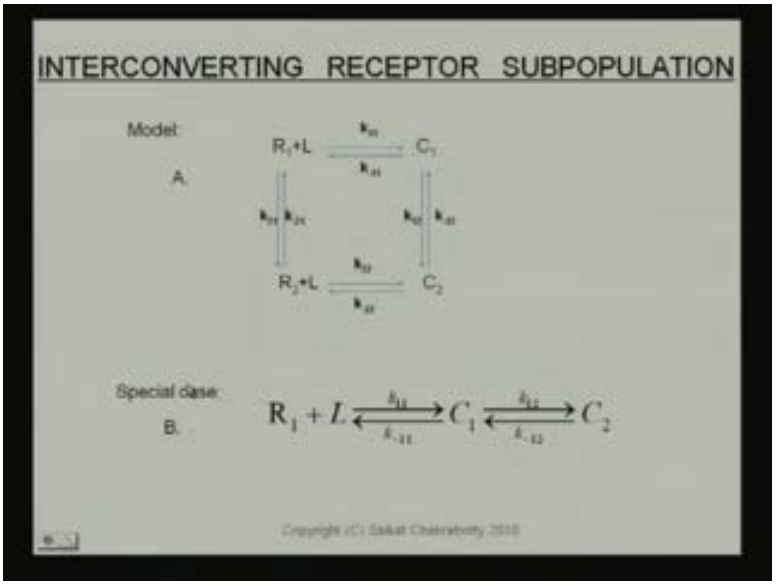

So, something like, where the receptors convert within, from two into each other, two

different forms. So, essentially what happens is that, in the previous part of the lecture what we talked about our receptor is that, work completely, independently of each other, when they bind to the ligand independently. What we are going to talk about now, there are cases where the receptors bind, but they do not bind independently. They bind; there is a independence between these receptors. Or in other words, the receptors populations are sub populations, which are interconverting to each other. And, what we mean when we say, we are, they are interconverting to each other.

Essentially it is a system of logical change; you know this is conformational change that is happening in the receptor. So, it is not the chemical composition of the receptors as such that are changing, but the conformational of the receptor, the conformational changes occurs in the receptor. And, what does the conformational change in tail; it essentially means that the rate constant, the dissociation and association constants. Or in other words, the binding rate constants would change for these cases. So, the slope, let us look at there.

So, interconverting receptor sub population; so, if you look at the screen what happens is that, often the receptor undergoes a conformational change, just as I said, after binding to the ligand. And, so, when we talking of two different receptor populations, how does this differ from what we had done before is that, what we had done before were two completely different receptors, which have affinity for the same ligand. Here, it is not that case. It is not two completely different receptors, but the same receptor which is undergoing conformational change. And, as a result, the dissociation and association constants are differing here.

So, the change, however does not affect subsequent ligand receptor binding. So, and, but there is a conformational change that occurs. And, in the most general case, the receptor is present as an interconverting sub population. That is what we are going to talk about in moral, today. And, so the most general case of receptors is present as an interconverting subpopulation. And, these subpopulations have differences in rates of dissociation and association between receptors and ligand. So, just as I said there, so because of the conformational change, what results; because of the conformational change is, essentially the fact that these receptors have different kinetic constants.

So, now, let us look at the model that we have over here; the interconverting receptor subpopulation model. So, as you can see over here, this is the most general form of the model R 1 plus l. So, R 1 is my receptor population 1; R 2, as I said is same, chemically the same receptor but just a conformation, R 1 with the conformational change. So, R 1 plus L forms the complex C 1. And, R 1 also forms the complex, changes to R 2 and forms the complex C 2. See, this picture may be slightly confusing. So, what I will do is I am going to write this differently.

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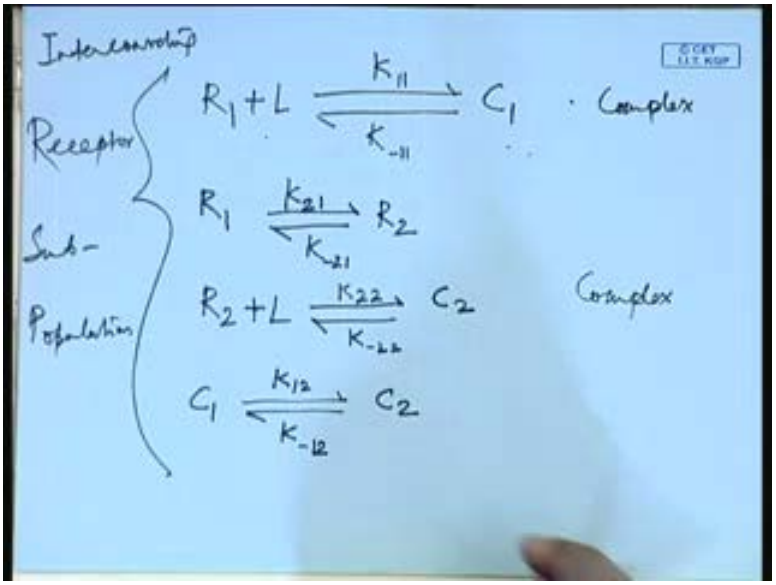

So, R 1 plus L K 1 1 K minus 11 C 1; so, R 1 is essentially reacting with the ligand to form the complex C 1. And, R 1 is going through a conformational change to form R 2. And then, you have R 2 plus L. So, again it reacts in the similar way as R 1. K 2 2 and K minus 2 are its rate constants to form C 2. And, these are the complexes; this is the complex, and this is the complex. And, it turns out, this is the complex themselves can undergo conformational change to rotate between each other. So, this is C 2 and the rate constants for this are K 1 2 and K minus 1 2.

So, this is the set of reactions; interconverting receptor, interconverting receptor sub populations. So, what is happening is that the receptor 1 is binding with ligand to form a complex and the rate constants, forward and backward rate constants for that are K 1 1

and K minus 1. Receptor 1 is undergoing a conformational change to form R 2. And, the weight constants for that are K 2 1 K minus 2 1. And, R 2 reacts with the ligand as it is, to form a complex C 2. And, C 2 and C 1 undergo conformational change again.

So, if you look over here, go back to the screen and look over here, you find that this is the way we have represented it. This is slightly confusing way of looking at it. And therefore, I drew it, but you can write this cyclical way. So, remember the only thing I want to point out here is that, it is R 1. When I draw these, these this this line, for example, the one the vertical line on the left; it is only the conversion between R 1 and R 2. And, L is not involved in this reaction.

Now, a special case of this is what you see over here; where the receptor is themselves do not change conformation, but it is the complex that changes conformation. So, R 1 reacts with the ligand to form complex C 1. And, it is C 1 that changes conformation to form complex C 2. And, the rate constants are K 1 1 and K minus 1 and K 1 2 and K minus 2. So, essentially what we have done is, if you take this cyclical model appear, if you cut out these two parts, these two lines over here and these two lines over here, you get R 1 plus L gives C 1, and C 1 gives C 2. So, you get this triangle over here and that is the special case.

So, what we will do now in the in the remaining part of this lecture is, essentially look at and model these. So, what we will do now is, to start with this. We will look at the special case B first; the reason being that, it is easier to model.

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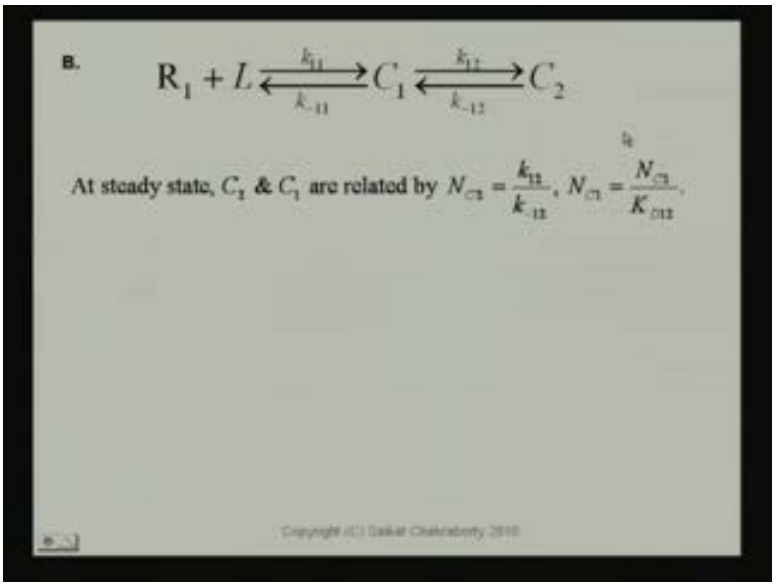

So, we will come back and look at A after that. But, essentially we will look at B first. So, in B, let us look at this. So, R 1 plus L, K 1 1 is the forward rate constant, K minus 1 is the backward rate constant. And, C 1 and C 2 is the complex, which undergo conformational change through the rate constants K 1 2 and K minus 1 2.

So, what happens when there is steady state? When there is steady state, you can relate the N C 2 and N C 1; that is the rate constant 1 and rate constant 2. Let me work out this for you.

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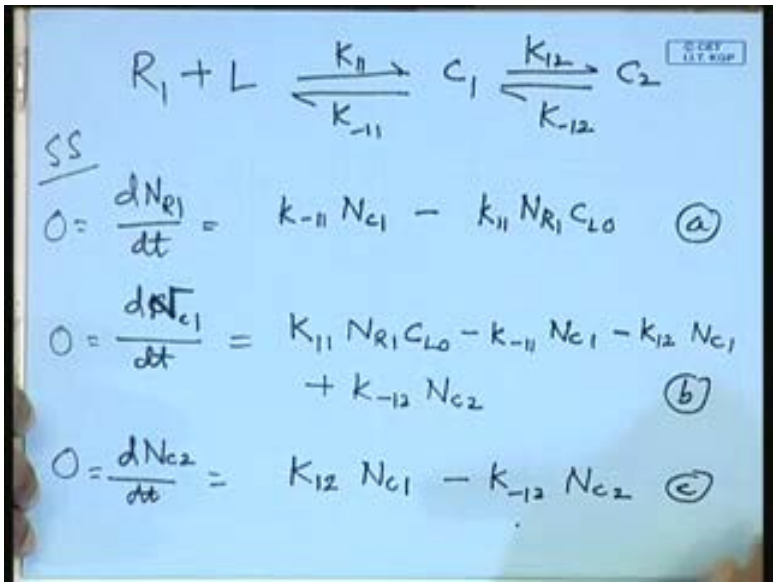

So, how this happens? Fine. C 1, K 1 2 K minus 1 2 and C 2. So, what is, let us write all these. So, d N R 1 d t, what is that equal, to that equals K minus 1 1 N C 1 minus K 1 1 N R 1 C L naught. Now, what would be my d N C 1, d N C 1 d t that equals… that is a little longer one; so, K 1 1 N R 1 C L naught minus K minus 1 1 N C 1 minus K 1 2 N C 1 plus K minus 1 2 N C 2. And, what would be my d N C 2 d t? That, simply equals K 1 2 N C 1 minus K minus 2 1 N C 2. So, at steady state, each of these would be zero.

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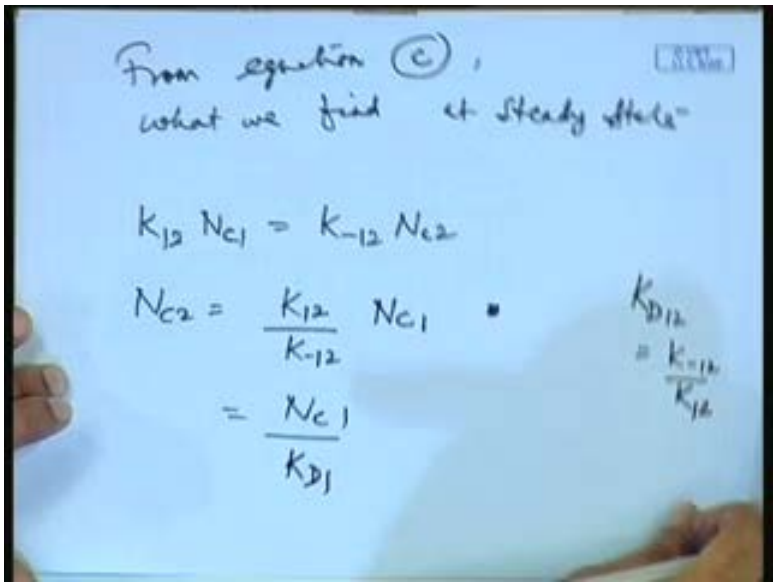

So, if that is zero, then from this equation, the last equation let us call this equation a, b and c. Then, from equation c from equation c, what we find is K 1 2 N C 1 at steady state equals K minus 1 2 N C 2. Let me go through this again. So, these are my set of equations that I wrote this are the, these are the model equations that dynamic model. Let us forget this steady state for a minute.

So, this is what I have and steady state model. So, N R 1 is being formed because of, so the N R 1 is being formed because of this reaction. And, it is been destroyed or removed because of the forward reaction. N C 1 is formed from this reaction as well as this reaction. And, it also removes because of this reaction as well as these reactions.

So, four terms here and whereas, C 2 is formed in the forward direction and destroyed or removed in the backward direction. At steady state, all these three have to be zero. So, what we do, we first equate the simplest one; the second one to be equal to zero. So, this is this is what we get. So, what we immediately get is that N C 2 equals K 1 2 over K minus 1 2 times N C 1. Or in other words, if K D 1 2 is written as K minus 1 2 over K 1 2, then this is written as N C 1 over K D K D 1. So, that is what I get.

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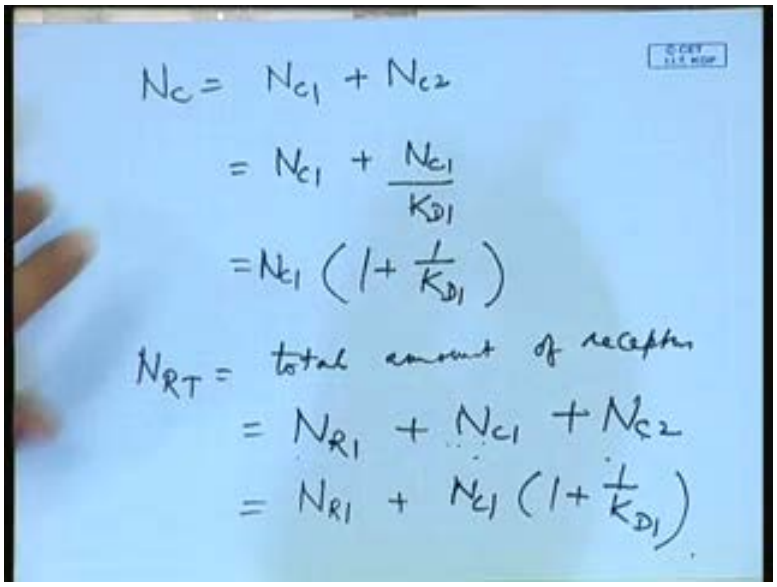

Now, what is my N C? N C would be N C 1 plus N C 2; which means that N C 1 plus N C 1 over K D 1. That equals, N C 1 plus 1 over K D 1. Fine. Now, what is my N R T? N R T is the total amount of receptors, which is N R 1 plus N C 1 plus N C 2. Or in other words, receptors that are completely free and receptors that are in complex 1 and receptors that are as complex 2. So, this I can write now as N R 1 plus. I have already covered calculated what my N C 1 and N C 2 together is which is N C 1 plus 1 over K D 1.

So, now if I go back to my equation over here, if I go back to my equation over here, so this has to be zero at steady state, also this has to be zero. So, what we can do is if I equate this to zero, I get a relationship between N R 1 and N C 1 straight away.

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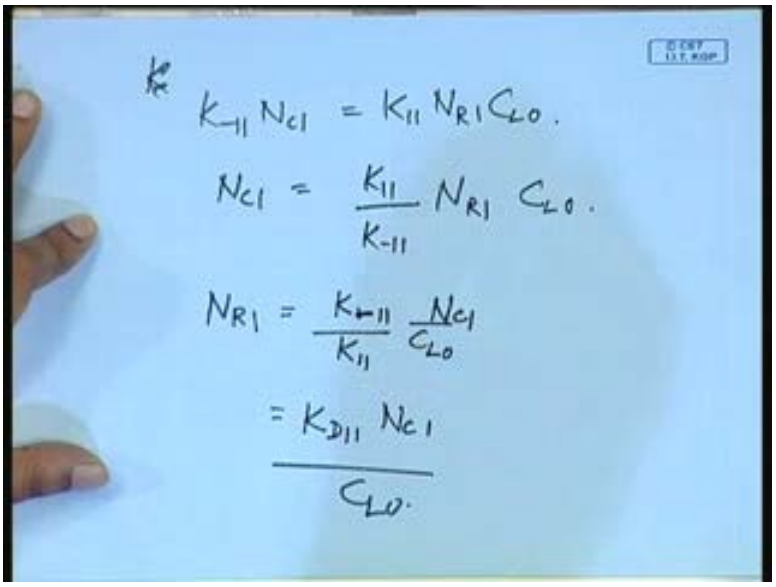

Let us do that. So, equating A to zero, so A is this equation out here. So, A is this equation. I am equating this to zero; which means that K minus 1 N C 1 equals K 1 1 N R 1 C L naught. So, N C 1 equals K 1 1 over K minus 1 N R 1 C L naught. fine. So, this is what I get. So, N R 1 equals K minus K minus 1 1 over K 1 1 times 1 over C L naught times N C 1 over C L naught. So, this I can write as K D 1 1, previously I had written K D 1 2. So, I can use this for K D 1 1, so C L naught. fine. So, this is one. So, N R 1 equals K D 1 N R 1 equals, just let me just put it over here like this yeah. So, N R 1 equals K D 1 N C 1 over C L naught and N C 2 equals N C 1 over K D 1.

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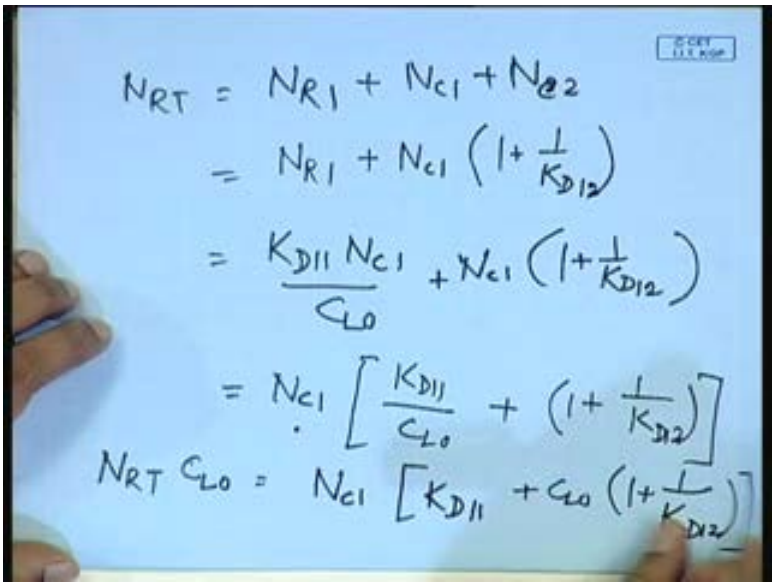

Now, what was my constraint equation? My constraint equation was that N R T equals simply equals N R 1 plus N C 1 plus N C 2 or I had written this as N R 1 plus N C 1 times 1 over K D 1. Now N R 1, I can write now from the from the previous equation, I can write is this N R 1 N C 1 over C L naught plus N C 1 over 1 over K D 1. K D 1 2. Sorry. This is K D 1 2.

So, now I can write this as N C 1 K D 1 1 over C L naught plus 1 over K D 1 1 1 2. So, this is what I get. So, this is my relationship between N C 1 and N R T. So, N R T times C L naught if I write, then it will be N C 1 K D 1 1 plus C L naught times 1 over K D 1 2. So, this is the relationship I have between these.

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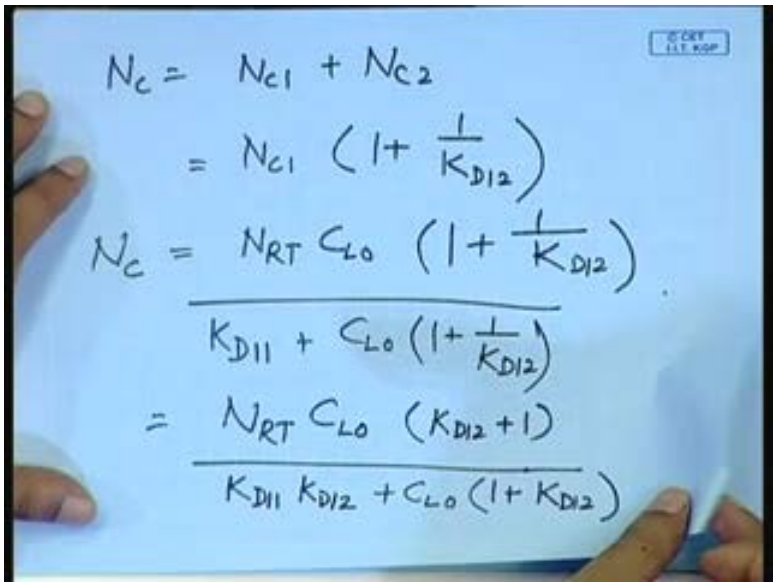

So, N C equals N C equals N C 1 plus N C 2; which I have written it previously if you remember, as N C 1 over 1 over K D 1 2. Now N C 1, I can now write as N R T over times C L naught divided by K D 1 1 plus C L naught 1 plus K D 1 2 times, this factor over here; which is 1 over K D 1 2.

So, this is my relationship that I have between these numbers. So, I can, one of the things I can do is try and convert this into a slightly more handle able form; which is K D 1 2 plus 1. And, divide this by K D 1 1 K D 1 2 plus C L naught 1 plus K D 1 2. I can write it like this or I can make it even simpler.

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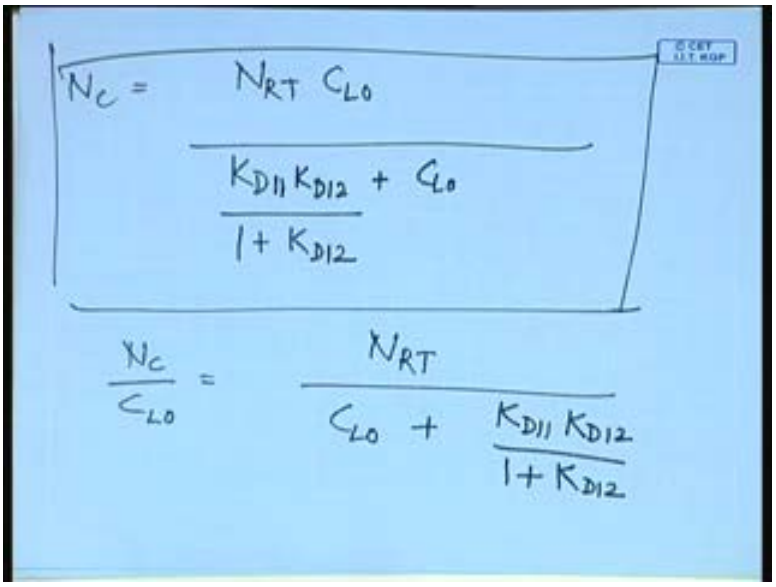

And, write this as N R T C L naught divided by K D 1 1 K D 1 2 plus C L naught, divide this by 1 plus K D 1 2. So, this is my final relationship between N C and N R T. So, if I want to draw my scatchard plot, which will be N C over C L naught; that would simply be equal to N R T divided by C L naught plus K D 1 1 K D 1 2 divided by 1 plus K D 1 2. So, I can draw a plot like this.

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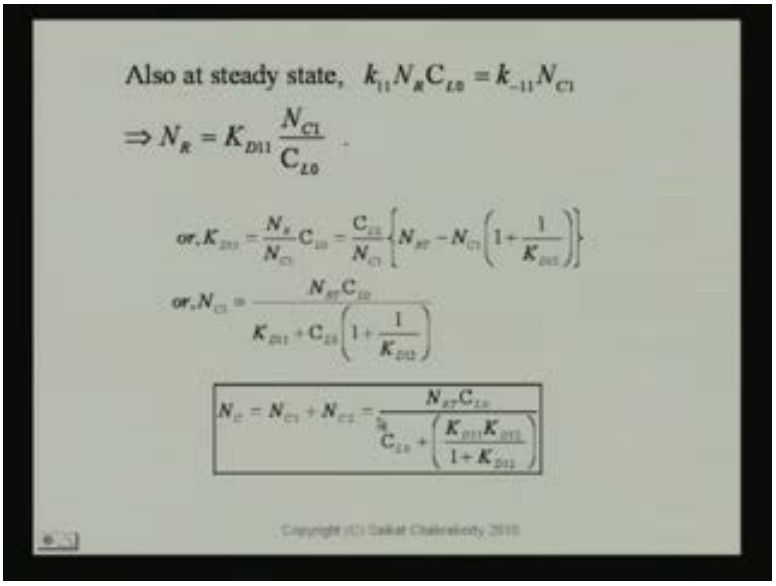

So, if I now go back to the screen, this is exactly I, what we derived; N C equals N C over C L naught equals N R T divided by C L naught over K D 1 1 times K D 1 2 divided by 1 plus K D 1 2. So, this is the plot that I want to obtain. So, why did I, why did I do it this way, why did I club club it this way because if I am to draw a plot of N C, so, what do I do? I can write this as K D apparent. My apparent rate constant I can write. So, if you look at this, it is even has the units of rate constant correctly. So, I can write this as K D apparent, which is K D 1 1 times K D 1 2 divided by K D 1 2.

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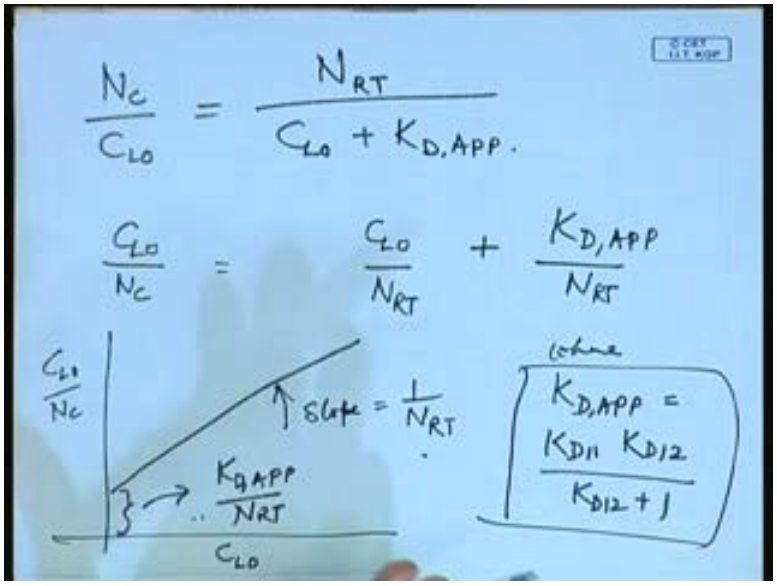

So, what will happen is, if I can now write my plot as N C L, N C over C L naught equals N R T over C L naught plus K D apparent. So, what I can do is, if I take a, if I can either, I can either do it this way, which is C L naught over N C equals C L naught over N R T plus K D apparent over N R T; where K D apparent is this number, which is K D 1 1 times K D 1 2 divided by K D 1 2 plus 1. Fine. This is my number. So, what I can do over here is, if I draw a plot of C L naught over N C versus C L naught, what do I get? I just get a straight line something like this. I just get a straight line something like this. And, the slope of this straight line is 1 over N R T; whereas the intercept is K D apparent over N R T.

So, why can, what I can straight away do is, from the slope I can calculate my N R T and

from the intercept, I can calculate my K D apparent. So, what was the whole idea of this entire process was to be able to we had many of constraints out there; K D 1 1, K D 1 2 and C L naught. And, C L naught is not a constant, but it is something that we can vary, but N R T.

So, what was the idea? The idea was to club these three constants that we had into two because two is something very measurable. And, write the equation in such a way that, we can plot it very easily. So, what we manage to do is, write the equation in a way where it is a straight line. And, we could just get the N R T from the intercept and K D; just get the N R T from the slope and the K D apparent from the intercept.

So, that was what we were trying, we are trying to do. So, this is the special case. The case, where the receptors themselves they are not interconverting, but the complexes are interconverting. So, what about the more complex case now? And, as I just said that this set of reactions could be replaced by this set of reactions are actually written by these set of reactions. And, what we do, you know, how can we look at this and model this now? This is slightly more complicated one the process that we are going to do. And, I probably, I am not going to have the time to work out the whole thing, but we will start to work out. And, the process that we are going to have to do is exactly the same process. So, this is, this is my plot, this is my, these are my sets of reactions. And, all we have to do we have to write balances for each of these R 1, R 2, C 1, C 2. And, let us try and do that, to start with.

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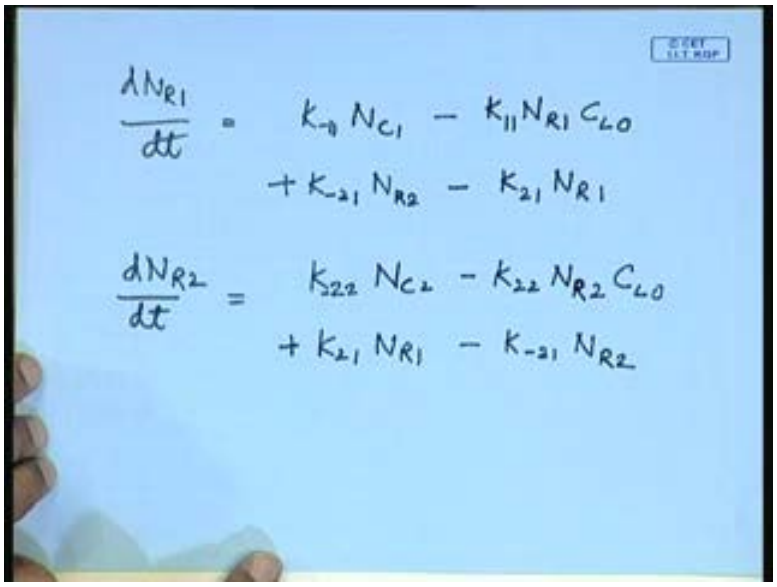

So, R 1 the balance from R for R 1, so it just for a second, I will keep this out here, so that you can write these set of reactions down. So, R 1 plus L K 1 a forward reaction K 1 1 backward reaction K minus 1 give C 1; R 1 forward reaction K 2 1 and K minus 2 1 gives R 2; R 2 plus L forward reaction K 2 2 backward K minus 2 2 give C 2 and C 1 forward reaction K 1 2 backward reaction K minus 1 2 gives C 2.

So, let us try writing the balances for R 1 now, first. So, R 1 is being formed by this reaction, K minus 1 1 N C 1 and is being removed or being depleted by this reaction N R 1 times C L naught. And, for another set of reactions and R for that R 1 is being formed from K minus 2 1 N R 2 and being depleted from K minus K 2 1 N R 1. Similarly, N R 2 d t is being formed for from K 2 K minus 2 two times N C 2 and depleted from K 2 2 N R 2 times C L naught. And, there is other inter converting reaction which is, it is being formed from K 2 1 N R 1 and depleted from K minus 2 1 N R 2. And, what about C 1 and C 2? That also, you have two sets of reactions.

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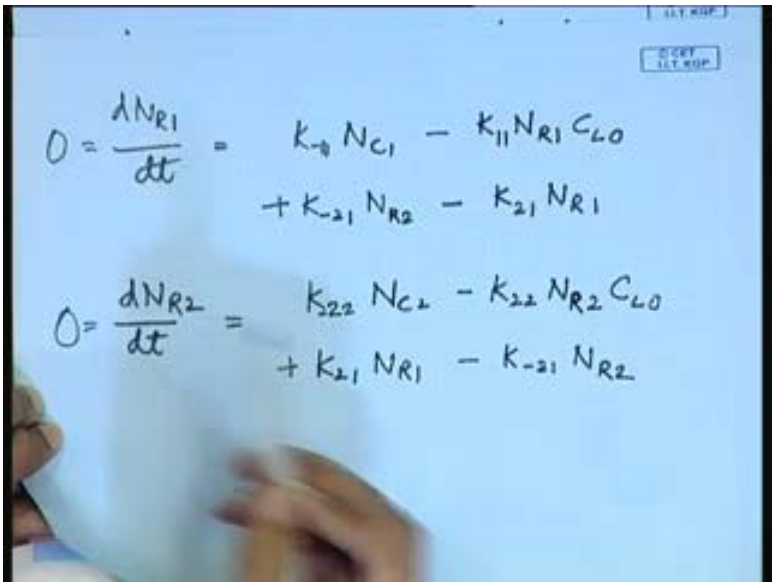

So, this is, this is their set of reactions for N R 1 and N R 2. And, if you look at C 1 and C 2, so d N C 1 d t equals K 1 1 N R 1 C L naught minus K minus 1 1 N C 1. And, then you have K minus 1 2 N C 2 minus K 1 2 N C 1. And, similarly we can write the balance for C 2, which is K 2 2 N R 2 C L naught minus K minus 2 2 N C 2 plus K 1 2 N C 1 minus K minus 1 2 N C 2. So, these are my four balance equations that I, that I write. And, what do we do with them? So, at steady state, I have to equate all of these to be equal to zero d t.

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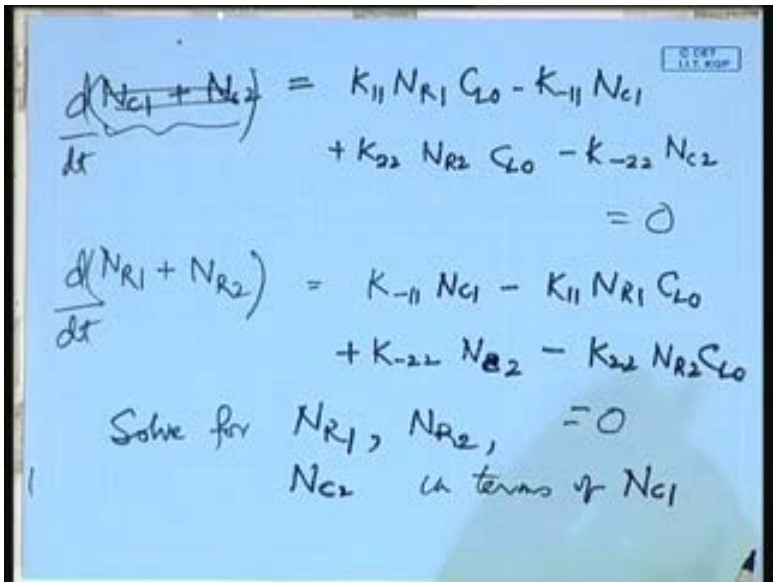

So, let us look at the total amount of complex that we have. So, this is my set of equations for N C 1. So what, let us look at the total amount of complex that we have, which is N C 1 plus N C 2. What is that number, if I add all the terms that I get over here? I get K 1 1 N R 1 C L naught minus K minus 1 1 N C 1 plus K 2 2 N R 2 C L naught minus K minus 2 2 N C 2. And, for N R 1 and N R 2, if I add the two equations N R 1 plus N R 2, just this is not. yeah basically- basically, this are adding these two equations. So, this is at steady state. This is and basically this is d by d t of this at steady state this equals zero. Similarly, d by d t of N R 1 plus N R 2 equals K minus 1 minus 1 1 N C 1 minus K 1 1 N R 1 C L naught plus K minus 2 2 N C 2 N R 2, sorry N C 2, minus K 2 2 N R 2 C L naught. So, this is what we get.

So, and this equals zero at steady state. So, what we have to do essentially is that, it is a little more cumbersome process. So, what we have to do essentially is that, we have to solve for N R 1, N R 2. And, let us say N C 2 in terms of N C 1, in terms of N C 1; let us say something like that. So, when we do that, then what we can do from here, what we can get from here is that, we can replace everything in terms of N C 1. Now, what was my constraint equation? If you look at these two sets of reactions that I have written over here, this is, let us go one by one.

So, this is N R 1 and N R 2, and this is N C 1 and N C 2. If you add these, all these four sets of reactions, what do I get? If I add all these four, what I get is d d t. If you look at these equations of N R 1 plus N R 2 plus N C 1 plus N C 2, the summation is d d t of that the summation is zero; which means that N R 1 plus N R 2. This implies that, N R 1 plus N R 2 plus N C 1 plus N C 2, they all, there sum come out to be a constant, which is N R T; that is the total amount of receptors present. Or, in other words, the total amount of receptors that is present is divided into the receptor 1, that is free receptor 2, that is free receptor 1 complex that has been formed and receptor 2 complex that has been formed. So, we go through these calculations and I am skipping the details of the calculations because we do not really have a lot of time.

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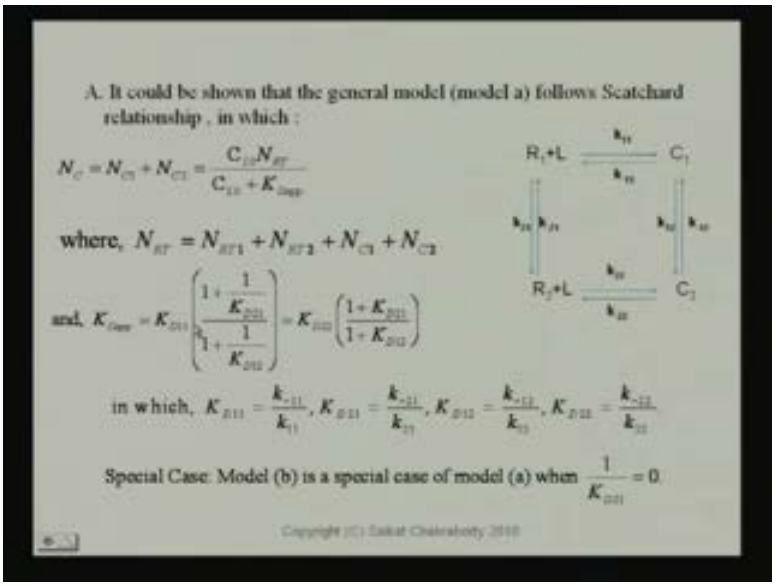

So, once we go through these calculations, if you look at the screen, what you find over here is that this constraint equation that I wrote. So, what you need to do? You have to keep substituting everything. So, you decide that you want to substitute everything in terms of say, N C 1. So, you convert N R T 1 as; write it as in terms of N C 1. Substitute here, convert N R T 2 and write it in terms of N C 2 and substitute here and N C 1 sorry and substitute here and write N C 2, also in terms of N C 1 and substitute over here. So, once you do all that, what you will find is just like in the previous case, just like in case B, as we did case B we did explicitly. And, here also, you will find just like in case of B

that N C could be written as C L naught N R T over C L naught plus K D apparent.

So, this is, this is what you can write. So, N C over C L naught is N R T over C L naught plus K D apparent, where K D apparent is slightly more complicated over here. It is K D 1 1 times 1 plus K D 1 over K D 1 2 1 over 1 plus K D 1 2, which could also be written as K D 2 2 times 1 plus K D 2 1 over 1 plus K D 1 2. And, these, all these numbers are written over here. So, K D 1 1 is the dissociation rate constant for the first case here; that is K D minus 1 1 over K D 1 1, K D 2 1 is K D minus 2 1 over K D 2 1; or in other words, the dissociation rate constraint over here, between the two receptor subpopulations. So, this is the way the receptors the changing conformation K D 1 2 equals K D minus 1 2 over K D 1 2, which is how the complexes are changing conformation. And, that is the dissociation rate constants for that, and K D 2 2 equals minus K D 2 2 over K minus K 2 2 over K 2 2 k. So, this is how the receptor, the second receptor is binding to the complex. So, this this is the thing.

Now, what happens is, what you had been able to do or we had been able to do through these processes. We had these one, two, three, four constants and N R T, of course the fifth constant. So, we have been able to reduce these five constants into two; or in other words, K D apparent, if you look at it involves all these constants. So, what we had been able to do in the process is, we had been able to reduce these four constants into a single constant K D apparent. And, why did we do that? The reason is again as the same reason that we had before, which is that we, it is very hard to be to be able to handle all of these. So, what? So, what we essentially have is just one equation over here.

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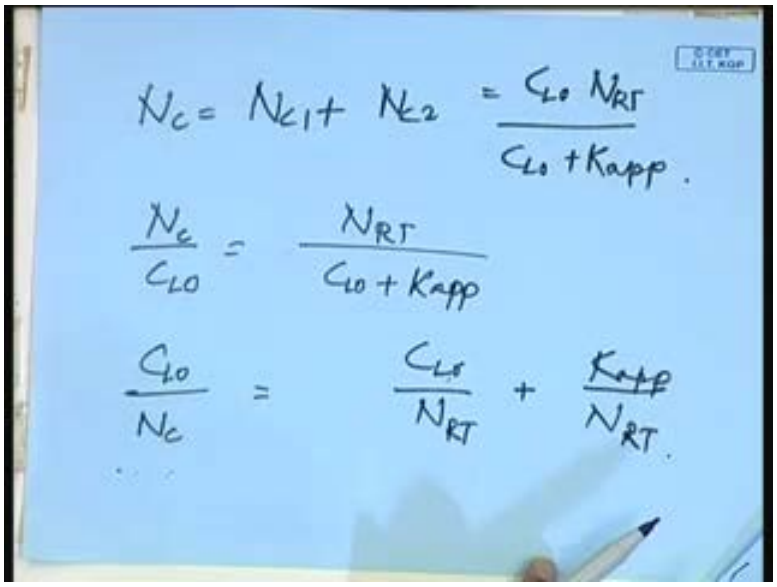

So, N C equals N C 1 plus N C 2 equals C L naught N R T over C L naught plus K D apparent. So, this is my equation. So, the advantage is N C over C L naught equals N R T over C L naught plus K D apparent. Fine. So, C L naught over N C equals C L naught over N R T plus K apparent over N R T. So, what do you do? You perform? take different values of C L naught, label the ligands and you perform your experiments for each of these sets and then you find out that, what what you essentially, you know what is the ratio of C L naught over N C for each of these sets and and measure it in terms of C L naught.

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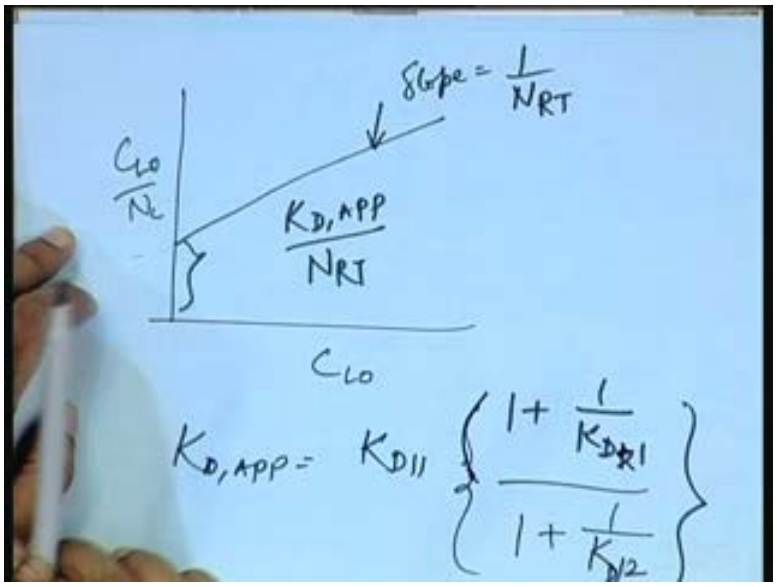

So, essentially this is your, if you want to plot it over here, this is you plot. So, the C L naught over N C and this is C L naught. So, just as I said that, this is going to be a straight line over here and the slope is going to be equal to 1 over N R T and the intercept is going to be K D apparent over N R T.

So, this is, this is essentially what we get and K D apparent over here, equals K D 1 1 over 1 plus slightly complicated formula 1 plus K D 1 2 divided by 1 plus K D 2 1 right here and divided by K D 1 2. So, this is my formula. So, what we essentially do? We conduct experiments with taking different amounts of ligands and figure out, how much is my total fluorescence that I get; that is a total amount of complexes that had been formed. And, we measure that and plot it against the amount of ligands that are there.

So, in case two, the case one, this is case one. And, case two, we did separately, but if we have been able to do case one, which is a lot more complicated case. And, case two, in case two would fall out, would be a natural fall out of case one and it would come as 1 over it come. And, when the case, for the case that 1 over K D 2 1 is zero or this case that is 1 over K D 2 1 is zero. In other words, K D 2 1 is much much greater than K D minus 2 1 that is or in this case, this case, this step is not there. So, if you go to case A, case B, you will see that what is case B? Case B is when the receptor populations, they are

themselves, they are not interconverting, but it is the complexes that are interconverting. And, when does that happen? When, there is no connection, no direct inter-conversion between the receptors.

So, if you look here, so what we are doing here is that, there is this reaction between the receptor inter-conversion and that reaction has to be absent. So, I think, we more or less looked at different cases over here. And, what you see on the screen right now is a summary of the in different processes that we looked at. So, if you remember, so this is I will, I will run you through very quickly. So, we started with the single receptor case. And, let me go through this very quickly and try and summarize what we have done in the last few cases. So, remember, this was the very important plots that we looked at just a second.

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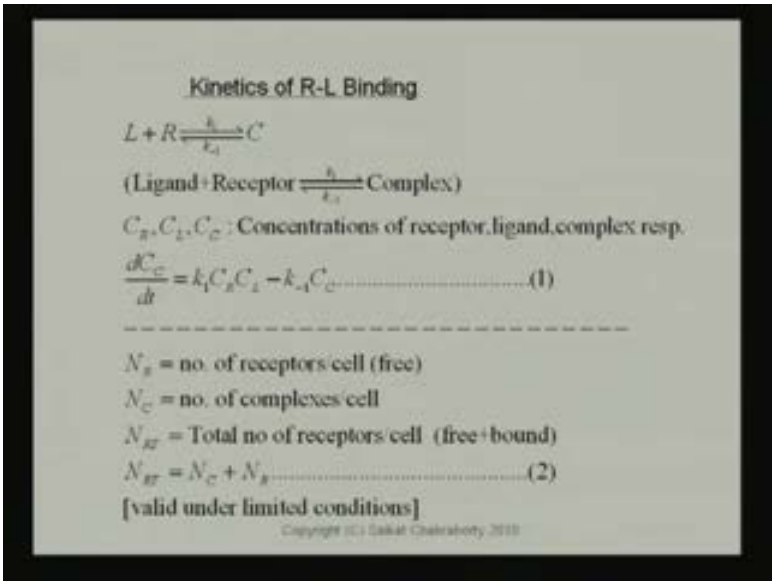

So, this is, this is where we started with. This is the single receptor ligand binding case, where there is a receptor binding binding to a single receptor, binding to a single ligand and we assume that, there is no receptor depletion out here.

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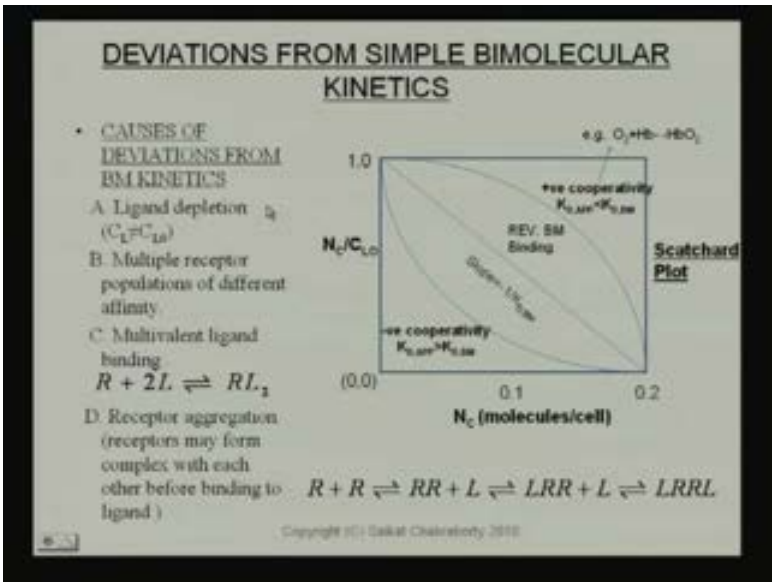

So, we got straight and a linear scatchard plots and stuffs like that. And then, we started to look at. So, this was the simplest case possible. And, for this case, the scatchard plot is linear, is a straight line. The scatchard plot is essentially the important plot that we will keep looking at here. And, N C over C L naught versus C L naught and we said that this is linear, but then we said that, there could be different deviations from this. And, what we had been studying in the last few lectures are these deviations from the scatchard plot.

So, the scatchard plot is linear, if the receptor, the if the ligand is present is is in excess and the receptor binds in the simple bio molecular kinetics.

Now, when the receptor there, they deviate from these, you have what is known as positive co-operativity and negative cooperativity co-operativity. And, that is what we looked at. So, you look at the scatchard plot over here and there is negative co-operativity and positive co-operativity and let me.

So, these are the different cases that we looked at different deviations. So, first one we looked at is ligand depletion. So, C L was no longer C L naught. It became a quadratic equation. We solved it and we showed how to do that. Next was multiple receptors

binding and what was the difference between multiple, what is the point about multiple receptors they were binding independently of each other.

So, two different receptors binding to the same ligand, but binding independently of each other. And then, we looked at cases where multiple receptors, they are not actually multiple receptors, but the receptors were changing conformations. As a result of which the rate constants were changing. And, they are essentially behaving like multiple receptors.

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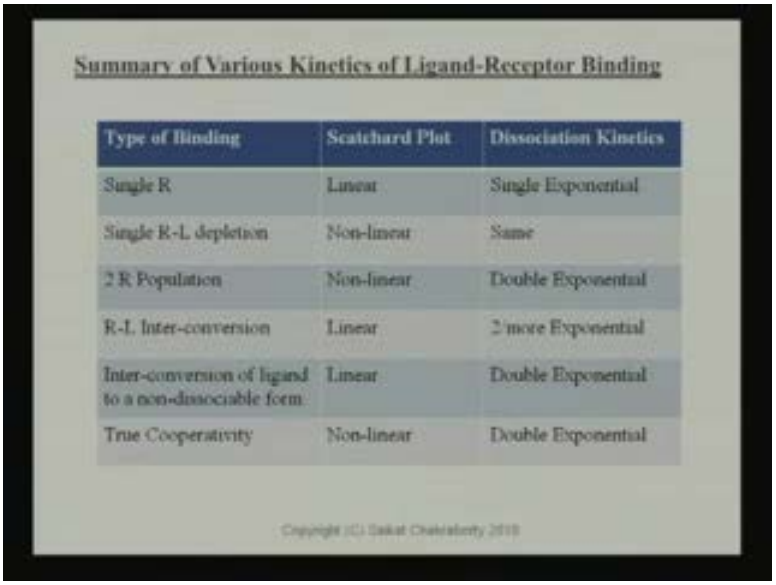

So, here is a summary of everything that we did in the last couple of lectures, last few lectures. So, single receptor, when we have a single receptor, the scatchard plot is linear and the dissociation kinetics is single exponential as it is written on the table, in the table out here. When you have a single receptor, but ligand is depleting, then the scatchard plot is non-linear and the dissociation kinetics is still single exponential.

Now, it is an interesting case of, next is the interesting case of two different receptor populations. And, the scatchard plot is non-linear and the dissociation kinetics is double exponential. And, we discussed this in the beginning of this class that, if the dissociation constant of one is very different, is very large as compared to the dissociation constant of

the other, we are in business and we can separate out the rate constant, the dissociation and the association rate constants of the two cases. Otherwise, it is very hard. Next, we looked at the case where receptor ligand inter-conversion and the scatchard plot is still linear as as we just showed. And, the dissociation kinetics is two or more exponential. And, this inter-conversion is what kind of inter-conversion? The receptor itself, receptor subpopulation itself changes conformation becomes another receptor.

So, because both these are chemically the same. They both bind to the same ligand, but the dissociation rate constants are different and they form different complexes, which can also interchange; that is interconvert through change of conformation. And then, there are other cases which we did not really study, but these cases I just want to go through quickly. One is inter-conversion of ligand to a non-dissociable form. And, and in this case, the scatchard plot is linear and the dissociation kinetics is double exponential as you see over here. So, inter-conversion of ligand to a non-dissociable form and then then there is true co-operativity in which case that, scatchard plot is non-linear and the dissociation kinetics is double exponential.

So, let me go and show you this over here, what we had before here. So, this multivalent ligand and these cases where receptor aggregations; so, these are other cases that can happen that is, one ligand is bind to two receptors. That is a straight forward case, but a little more complex cases, the case of receptor aggregation which is that, two receptors R plus R together form an aggregate. And then, this binds step by step with a ligand. So, essentially you form L R R L, which is two receptors binding to two ligands, but the fine bond bind as an aggregate; which means that two receptors bind to one ligand first. And then, this ligand with the two receptors binds to the second ligand to form LRRL.

So, again the kinetics here is going to be little different. The reason this is going to be little different is that the kinetics is going to be little different is because the dissociation, association rate constants are varied over here. And, this is the case of co-operativity and I, if you remember at the beginning of this course of this chapter, I gave you the example of haemoglobin molecules, four hemoglobin molecules binding to oxygen step by step and this is the very parallel example of that. So, two receptors binding to one ligand first and then the two receptor one ligand complex binding to the second ligand and forming

two receptor, two ligand complex.

So, there is the co-operativity out here and there is positive co-operativity. This is similar to the haemoglobin oxygen binding. And then, what will happen is the rate constants will be different at each of these steps and it, they would be different. So, two receptor binding to two ligand is different from the rate constant. The simple case of one receptor binding to rate constants would be different from one receptor to binding to one ligand.

So, these are the different kinds of receptor ligand bindings that we talked of the kinetics of this. Now, where does these kinetics come into play, what is the, what are the real physical processes where these kinetics are very important. And, I had talked a little bit about this. And, the one particular case that we are going to look at and we are going to look at with respect to particular disease, which is familial hypercholesterolemia in the following lecture, is receptor-mediated endocytosis.

So, receptor-mediated endocytosis is the very important process. And, it is very important for several biological and physiological processes. And, this is where the kinetics come in. And, the disease that we are going to talk about familial hypercholesterolemia is a genetic disorder. And, we are going to look at why this genetic disorder happens. It is because of some kinetic disabilities that is, some kinetic rate constants is lower than what they should have been and or some other reasons. You know the complexes not being formed properly or not enough ligands or not enough receptors, what is the reason?

So, this is a very practical and interesting example and application of this theoretical study of receptor ligand binding. So, we did a purely theoretical study till here of receptor ligand binding, but we are going to apply it in the following lecture to different diseases and then name. Main thing that we are going to focus on is the process call receptor-mediated endocytosis. With that, I will stop to today and we will talk about receptor-mediated endocytosis in the following lecture. Thank you.