Biochemical Engineering

Prof. Dr. Rintu Banerjee
Department of Agriculture and Food Engineering
Asst. Prof. Dr. Saikat Chakraborty
Department of Chemical Engineering
Indian Institute Of Technology, Karagpur

Lecture No. # 30

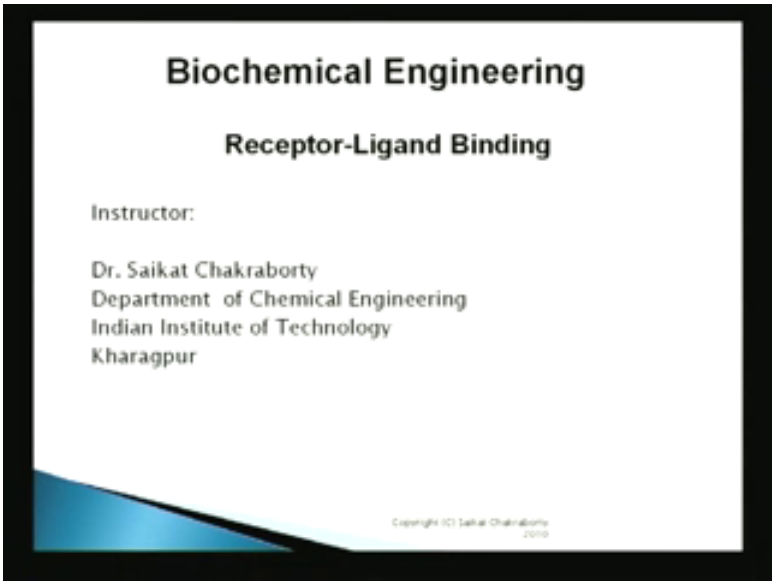
Introduction to Receptor-Ligand Binding

This lecture series on biochemical engineering and it is titled receptor ligand binding. Receptor ligand binding is something that you should be curious in you know physiological engineering so to say and a large fraction of the biological processor biochemical processes that occur in a human body or any kind of physiological systems are resultant from receptor ligand binding. So, what is this process of receptor ligand binding that is one of the things that we will study and then we will study with respect to a one particular disease which is a well known disease and it is kind of very as from prevalent in India.

So and with that we will just use the disease as an example to study the receptor ligand binding, but initially will try and understand the mechanism of receptor ligand binding, the kinetics of receptor ligand binding and how the kinetics effects certain issue with receptor ligand binding. Then, we look at a physiological aspect or a pathophysiological aspect of it that is some of the diseases.

So, as I said that many of the processes biochemical much of the biochemical, most of the biochemical processes rather are result from receptor ligand binding or in other words the result receptor ligand binding plays an important and in most cases a limiting role in these biochemical pathway. Having understood that, we also figure out over time that we have figure out over time of the last half centuries. So that many of the diseases which is genetic especially are due to the fact that there are some problems or you know in adequacy or problems or deficiencies in receptor ligand binding.

(Refer Slide Time: 02:02)

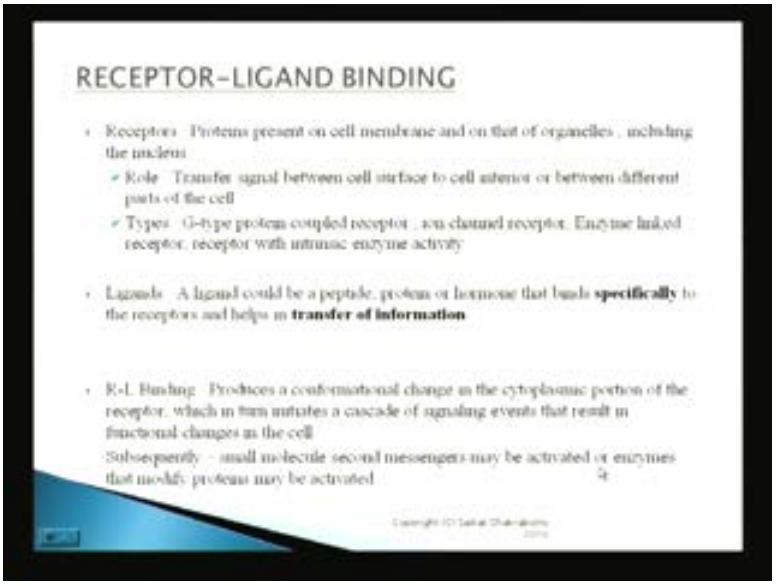


So for example, if you genetically inherited disease from your parents or your grandparents it could be that it is it has to do with the a fact that you know inherited a gene which somehow impedes the kinetics of a receptor ligand binding related to that biochemical pathway, as a result that disease is you know manifest in you. You see what I am saying.

So, it is not necessarily that all diseases would be manifest because of a certain biochemical pathway, but for example, diseases like high cholesterol or diabetes or things like that in many of these diseases has receptor ligand binding as a part of it. So, we will do first look at receptor ligand binding as a phenomena first as a mechanism first and then we will look at one particular disease which is familal hypercholesterolemia. That is again a genetic disease and we will try and understand that what are the factors that lead to the genetic disease and it will it turns out that we will figure out that much of it has to do with improper receptor ligand binding. You know in receptor ligand binding that should have been there, the reason the way it should have been.

So, having said that so let us start the chapter essentially. So, it is called receptor ligand binding and it is a you know 6-7 lectures we will have on this is probably longest of the chapters that will do.

(Refer Slide Time: 03:12)



So, what are receptors you know before we try and understand receptor ligand binding. What are receptors and what are ligands? Receptors are proteins that are present in cell membrane and organelles including the nucleus. And I will show you pictures in may be a less than a minute of how the receptors look. So, these are proteins which are present on the cell membrane and that of the organelles and what is the role of receptor ligand binding or receptors so to say. It is to transfer cell signal from the cell interior to the cell exterior. So, these are in a way these receptors are in a way, connects between the interior of the cells to the exterior of the cell.

Now, one of the things that I keep telling you know probably not in this lecture, but in another classes is that there is something called an inherent wisdom of the cell. The cell itself has cell protecting mechanism just like the body itself has a self protecting mechanism or self defense system, either a self defense system or a self protecting mechanism so you are in an environment for example, where there is low oxygen and you would be instinctly ,you know instinctively you will be guided by your body to move away from that environment to an area where there as higher oxygen or where there is a fire out there instinctively even if you are you know if you are not thinking, your body would instinctively take you away of that.

So, there is the self protecting, self defense mechanism of the body. Similarly, each cell is a full fledged all most like a full fledged individual because you know come to think of

it we the cell is a building block of the body and these are the whole of life on earth itself started with a single cell not with multiple cells.

So, come to think of it we are just a (( )) of cells. So, and there is each cell of the wisdom is wisdom of its own which is to protect itself. a number one on the first priority is to protect itself and b is to perform the function that it is suppose to perform. When it cannot perform the function that is supposed to function, then it dies you know and in this process I think I have spoken about that sometime earlier.

So, the process what I am trying to talk about is that so the cell the cell has a whole has this mechanism of self defense and self protection and for that it needs to function on its own, but again the cell, one particular cell needs to act as a part of an organization and that organization you can call it a tissue, an organization of tissue is called organs and an organization of organs is called the human body. So, the cell has to function from the level of a single cell to the level of the tissues to the level of the organs to the level of the human body.

So, how does it communicate with the exterior? It is not enough to for us for a for a body for example, you want to preserve your body and preserve your system, at the same time you need to communicate with external world, you need to communicate with your friends, with your teachers, with your family for different things because at the simultaneously we are you know self preserving system at the same time we are part of a larger world.

So, there is a necessity to communicate and there is a necessity to preserve oneself. The reason to communicate is sometimes for example, the hydrogen ion concentration inside the cell has grown to a level say higher than such that p h is higher than you know 7 point is lower than 7 point it has become more acidic than it supposed to be, something like that. So, then the cell needs to communicate to the external environment to kind of balance this, the cell needs to communicate with the external environment to kind of balance this so that the p h is decreased within the cell because acidic p h is not good for the cell.

So, these kind of communications are not just necessary to work as a group, for the cells it is not just necessary to work as a group, but it also necessary for the cells to preserve itself and the receptor ligand binding or receptors themselves act as a very important

source of that. So these are the sort of you know coat and coat the neurological not really, but the neurological connects between the cell and the outside environment.

So, they are the ones who allow the cell to communicate with the outside environment. And these if I go to the screen now, so these are receptors are essentially proteins that are on the cell membrane. It could be on the organelles also for example, why on the organelles because if this nucleus wants to connect or communicate with the cytoplasm then the receptor in that case is going to be on the surface of the nucleus, taking out of the surface of the nucleus instead a inside the cytoplasm otherwise if the cell is trying to connect with the outside environment then the receptor is going to stick out from the cell membrane into the outside environment. I will show you a picture how it is on both sides of the membrane and the major job of this receptors are is to transfer signal between the cell surface and the cell interior or between the different paths of the cell.

If it is a case, if it is between nucleus and the cytoplasm then between the different paths of the cell. If it is across cell membrane then it is transfer signal between the cell surface and the outside and what are these receptors? These receptors are g protein type receptors, ion channel receptors, enzyme link receptors, receptors with intrinsic enzyme activity you have read some these you know I think the protein receptors, ion channel receptors we had discussed this in a previous course, but so these are so all of these means are essentially to communicate between the external and the internal environment.

For example, the protein channel of the ion or the ion channel receptor for example, you know if you want to throw away certain ion say sodium or say potassium or say hydrogen then this receptor would get that signal to the system saying that the ion channels have to be opened out and you want to throw out this particular ion. So that is a function of these receptors. Now, what about ligands? So, ligand could be a peptide, a protein or a hormone that binds specifically to the receptor and helps in transfer of information. So, it is like you know mechanism where the ligands, so the receptors just sticking out into the cytoplasm does not make much sense. It has to bind with the ligand and transfer information, but look at 2 words which are in bold over here, transfer information and the other one is specifically. So that is important which means that it is like a lock and key mechanism. So, a specific receptor will bind to a specific ligand. That is the whole idea. So there is a lock and key mechanism just like in the case of enzymes, you know enzymes will bind to particular substrate so similar kind of thing. And now the

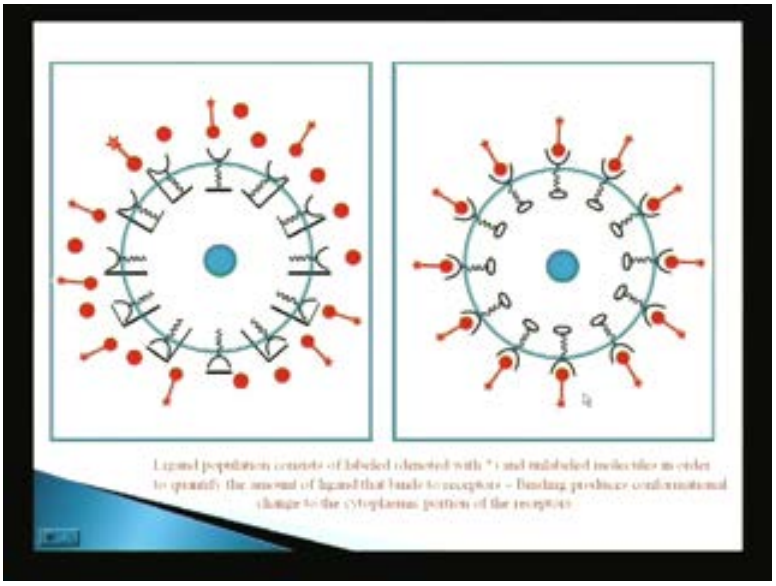
what is the idea of the receptor ligand binding? So, we talked about the receptor, we talked about the ligand. Let us talk about the binding that follows. So, how does this biding occurs? Similar, very similar to the enzyme binding to a substrate.

So, what happens is when a receptor bind ligand binds to the receptor and I will show you a picture in a minute it produces a conformational change you know this I would not be able to show, but you can sort of imagine it. So, it produces a conformational change in the cytoplasmic portion of the receptor which in turn initiates the cascade of signal in facts that results in functional changes in the cell.

So, whenever these see you want to transfer a signal that is your job. That is the reason we work do, you are doing this binding. Now, you have a set of ligands which will bind specifically to the receptor. Now, as soon as the receptor binds to the ligand there is conformational change that happens in cytoplasmic pattern part and I will show you these two part and the cell signaling starts, the process is initiated.

And then the, after this process is initiated small molecules and say small molecules of which is secondary messengers they may be activated and enzymes may modify the protein also. I mean this is the something that happen after the conformational change occurs, after the cell signaling process starts, you can have secondary messengers you know in implementary system you have certain kind of messengers t cells and you know different kinds of secondary messenger which are activated and you can start the process which are related to secondary information passage not the primary one.

(Refer Slide Time: 11:29)



So, if you look at this picture over here. So, this look at this. So, you have over here what we have is a set of ligands which are these are labeled ligands and these are general ligands. So what is the difference between a label ligand and general ligand? The difference is that I have just taken here, I know that this receptor is going to bind to a particular kind of ligand and I labeled that ligand and I mix it up with other ligands or other ligands are also there automatically you know generally they are there in the system.

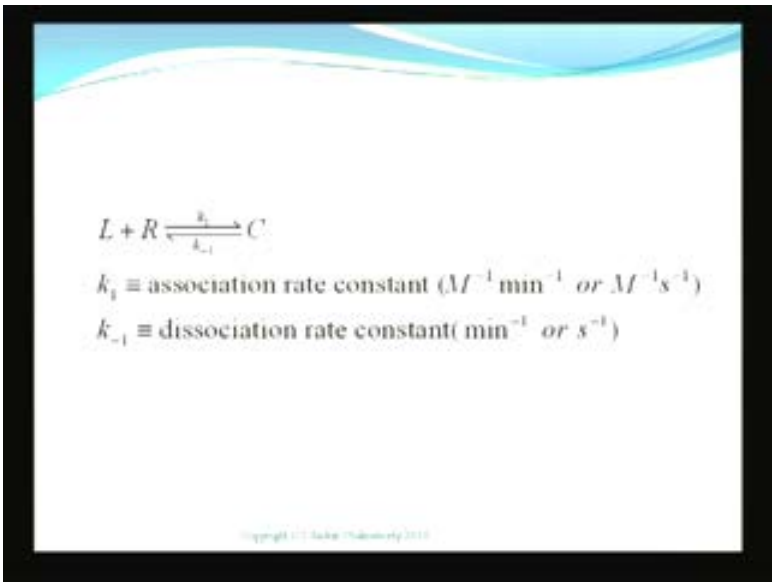
So, I have no control over this. So, the label ligands are the ones which is specific to the particular receptor. Now, you see what is happening over here look at this picture and look at this picture. So, at the end of the binding process you see that the label ligands only and the label ligands only have bound to the receptor the reason is specificity because they are the ones that are allowed. Also notice this receptor over here, this is how receptors are always drawn by the way you know when you draw receptor on you copy this is how you draw cup, half a cup shape over here and a spring like structure the and tail.

So and the tail is in the cytoplasm and the cup like structure is ejecting out of the cell membrane and the cup like structure is such that the reason the cup like structure as I said this is a lock and key mechanism, so this receptor can ligand can come and sit over there. Now, look at this what is happing this is a nucleus of blue one out here. Look at

what is happing in the cytoplasmic part of the cell from here to here, there has been a confirmation change which is denoted by this.

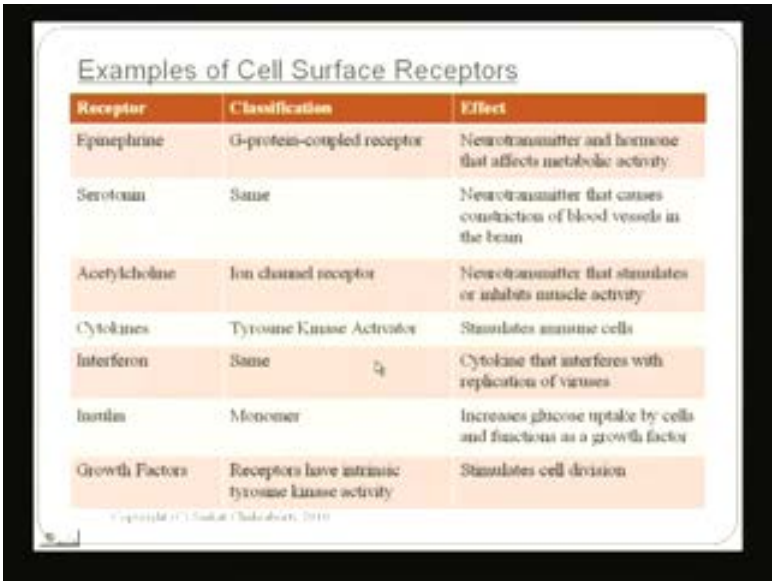
So, that is a thing so ligand population which consist of the label ligands and unlabeled molecules in order to quantify the amount of ligand that binds and binding produces a conformational change to the cytoplasmic proportion. So, the next thing that we want to do is try and quantify this in terms of the kinetics, so what do you think could be the kinetics of binding . It is not going to Michaelis Menten, but similar to the enzymatic mining, it is not going to a Michaelis Menten the reason being you know the steps of the pseudo, the pseudo steady state reaction equation and all those steps are not exactly the same but the equation in general looks like that. It is the kinetics actually lots simpler.

(Refer Slide Time: 13:50)



So, let us talk about the kinetics. So, Ligand is denoted by L always, R receptor and C is a complex that that it is formed. So K 1 is a association rate constant which is a second order rate constant as you can see molar inverse times minute inverse or molar inverse times second inverse and K minus 1 is a dissociation rate constant which is minute inverse or second inverse.

(Refer Slide Time: 14:23)



So, these are examples of cell surface receptors that is there in the human body and you know some of these for example, let us go through them Epinephrine. Epinephrine is a well known neurotransmitter. It is a pretty well known neurotransmitter and it is it regulate from metabolic activity, you know it regulates hormones and that kind of make metabolic activities and it is a as I tell told that there are different kinds of receptors and if you can see in middle column it is a G protein couple receptor. Serotonin. The next one is Serotonin. In the serotonin is a neurotransmitter that control causes a constriction of blood vessels. It is a very well known. Have you heard of serotonin dopamine? You have not heard of serotonin and dopamine.

It is a very well known neurotransmitters you know for example, if you know if you have a friend with some depressed or something what do you gift, what is something that you typically give to that friend? Something to eat? What kind of things to eat? No idea, what you get to your friend who was depressed. Chocolates, typically that is what is given at least from the.

So, why is that? Because chocolates has the chemicals which if you look here on the screen which stimulates the production of serotonin and dopamine and these are so any at any point of time so feeling sad just go by eat some chocolates that helps. You know it is in terms of cholesterol or whatever may not be very good, but it is well known the chocolates has a lot of neurotransmitters in there that I mean has things chemicals that

stimulate these neurotransmitters rather serotonin and dopamine and as a result these are and so it is these are a produced more in your brain and goes into the blood stream and constriction of blood vessels in the brain, as a result you feel this state of happiness or joy or whatever. So, this whole you know every emotion that you feel is essentially a chemical reaction, come to think of it quiet sadly. So, your whole expression you know when you feel good about something, when you feel happy about something essentially being just the fact that serotonin and dopamine are being reduced to, are being produced in the right quantities. Now, if you do not have them in the right quantities you feel depressed or sad or low, if you have too much of them you feel euphoric.

So, these are the chemicals you know these I am sure we have been taught in the other part, but these are the some of the chemical supplies the serotonin and dopamine. Know these actually a chemical supplies. So similarly, Acetylcholine is an ion channel receptor and it also is a neurotransmitter. So these 3 are the major chemicals that work you know a neurotransmitters that work in the brain.

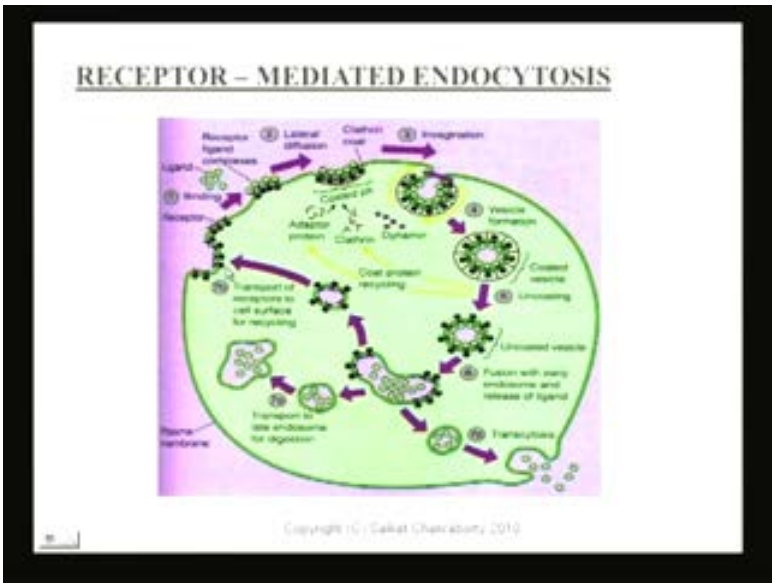
So, Serotonin, Dopamine, Dopamine is not listed here and Acetylcholine. So any sort of feeling of goodness that you have at all points of time are a permutation and combination or a or a total production of these 3 things you know. It is not God's gift to you, happiness is not God's gift to you, it is just these 3 chemicals Serotonin, Dopamine and Acetylcholine working together to produce that emotion. Similarly, other kinds of emotion are produced as I said sadness is produced by the you know by a depletion of these chemicals. Now, next so these kind of form the same group. Epinephrine is one that stimulates hormone activity and this is also may you know important. Epinephrine also kind of takes part in this.

So, this is kind of one block you can think of. This is a G proteins or this is a G protein this ion channel, but these are one block of neurotransmitters that govern a lot of emotions and lot of feelings in the brain that you have. Cytokines is a different kind of receptor, it is not this neurotransmitter group. Cytokines I know I am not sure if I talked about it, but Cytokines are essentially immune cells.

So, monocytes and macrophages these all belong to Cytokines and derives did I discuss the effect of cytokines and things like that, maybe I will in may be. Did I discuss before the effect of cytokines how thing macrophages and stuffs like that? I think we will do

this in this course also where I can give you a little briefing, a quick little briefing and probably I have a picture out here, I can show you the picture and yeah I think.

(Refer Slide Time: 19:25)



So, this is receptor mediator endocytosis, but similar is a process for this way this see I have a better picture, so this is a probably the best picture I have. So Cytokines do not really work the exact way, but this endocytsosis process it is the same. So, if you do not think of this is a cell in general, but if you do not think of this is a cell. If you think of this as a as a Cytokine then this is exactly what happens. So the Cytokine will form these parts, so Cytokine is like say a macrophage, will form this path which are known as invagination.

So, a part of the Cytokine deforms itself and see you have a so deforms itself like this, like a mouth should I have been going to the details later and forms this kind of thing. You can see here this kind of structure out here. So, why does it form this kind structure? Because the bacteria, so these are immune cells and what so when the bacteria attack. Let us talk about so what happens when you have bacterial attack you know for example, you inhale you know you breath in certain bacteria or you grabbed something which had a some bacteria something like that, let us talk about the simplest case where you inhale, somebody sneezed in front of you and you inhaled certain bacteria.

Now, if your immune system is good then you are not necessarily going to fall sick. If your immune system is not good at that point of time, it is a function of time also then

you might fall sick. So, what happens when the when the bacteria enters your body? What happens is that it goes through the larynx you know you breathed it and it goes through the larynx, pharynx, trachea and so on and goes in to the lung. Now in the lung what you have is that you have the cells on the on the lung and on these cells you have around 7 macrophages which are there from these cells now per cell per cells. So, it is a huge and on the alveoli is actually so each alveolus actually will have 7 macrophages. So it is like around couple of billion macrophages I think in the lung itself.

So, each macrophage if I now can go to the screen, so each macrophage is like a cell like this. So, what happens the bacteria comes and say the bacteria's entered your alveolus. Alveolus is a little sacked like spaces inside your lung, entered it and it is out here, somewhere out here.

So, then the process starts. The process is first step in the process is that the macrophages which is small in size typically increase in volume a lot 4 times, 5 times increase in volume. First thing is the increase in volume, as a result the energy requirement goes up. So, it changes the macrophages changes stage from what is known as peace state p e a c e state to war state. So, the body so your the process start with a again a receptor ligand binding, so there are something called t cells with there are some receptor ligand binding that goes on and they sends these bacterial that are there in the system.

So, once if cells is bacterial that there at the system, they send some signals to the central nervous system saying that that this process, there is a bacterial attack in the system. Now, once the bacterial attack has been sensed by the brain, by the central nervous system then it sends out signals to the, so these are all these signals what through neurotransmitters and you know essentially receptor ligand binding, so that is the whole job of receptor ligand binding.

So, these signals are sent to back to the lung saying that there has been attack so it is like attack on a country for example, and a country goes from the peace state to the war state. So what does it do? It deploys soldiers to the boundaries of the nation. So similarly, the brain tells the lung that there has been attack on the system a bacterial attack on the system or a viral attack, it could be a viral attack does not have to be bacterial attack on the system and we need to deploy soldiers.

So, what do you mean by soldiers? The soldiers here are the immune cell the Cytokines. In this case in the lungs a different kind of cytokines you have in different parts of the body. So, the Cytokine that is important in the lung is called macrophages, in the liver or the kidney they are also there, also you have macrophages, on the guts and you know the other kinds of Cytokines as well as I said monocytes and so on in the blood. So, the Cytokines are alerted. Now, once the Cytokines are alerted then what happens they say that the brain tells the Cytokines that increase the volume because unless we have big in volume, so they cannot beat the bacteria or the other virus. Now, when you increase the volume you need a lot of energy to increase not just to increase the volume, but to sustain it why because an increased volume just as we read in the in the cells cell growth process, an increased volume will necessitate increased metabolic activity.

So, increased metabolic activity means increased use of energy and have you ever noticed that if you have a bacterial attack even before you have a fever on anything you start to feel very weak or viral attack, either of these two attacks. Even before you got a fever you start to feel very weak. Why do you feel weak because your energy is now being diverted to your immune system.

So, your immune system is being pumped up while the rest of the body kind of takes a setback. So the doctor would tell that you know eat well if you if you have fever then eat well you know eat enough glucose or stuffs like that, drink enough water especially water that has carbohydrates in it or you know the this you know these kind of I mean is saturated with a glucose or some kind of carbohydrates or other. So, you do that so that you provide metabolism to the Cytokines. Now the Cytokines will expand in volume.

Then, let us now go back to the states. So, once it expands in volume you form this invagination. Why does it form these invaginations? Because it want to engulf the bacteria. So, the bacteria so around the place where the bacteria is there it forms these things and if you have 7 for example, 1 alveoli has 7 macrophages. So these 7 the these macrophages will form invaginations and 1 macrophage can form multiple invaginations. It forms these and it waits for the bacteria it is like you know trying a putting a fly on and waiting for the fish.

So, it waits for the bacteria the virus to come here and as soon as it comes here it entraps it. It entraps it like this and then it forms and then it joins its both you know both, it is

like a mouth of the macrophage it kind of eats the bacteria up, closes its mouth and this is what is formed. You see here this in the where the arrow is, this is what is formed out there. So and the bacteria's entrapped now here, now what? What do we do? You have a living bacteria inside a macrophage, the macrophage cannot afford to keep that bacteria just like that out there for a long time because the bacteria might start to multiply and infect the macrophage itself.

So, what would it do? What do you think it should do? So, that is what it is going to do, it is going to acidify. Why is it going to acidify? Because essentially what it needs what you do when know when you want to kill bacteria in the drain or anywhere what do you do use?

Either acid or phenyl or something like that essentially at the end of the day what you use is a bleaching agent which is what either chlorine or hydrogen peroxide a bleaching agent. So, the cell the macrophage also uses a bleaching agent. The bleaching agent it uses is hydrogen peroxide actually and hydrogen peroxide is not there in this system. So, what it does is it produces hydrogen ion and the hydrogen ion then reacts with the water to form hydrogen peroxide. So, what happens here is that this is known as a vesicle once it is kind of taken in and then it acidifies out here which is that it pumps in hydrogen ion. Where does it get the hydrogen ion? The hydrogen ion is present inside the cell. So, it keeps pumping in hydrogen ion and then there is you can say that yes there is going to be a depletion of hydrogen ion, yes, there is going to be then these other ion channels are going to open up and hydrogen ion is going is come in from outside and then it is going to pump in hydrogen ion inside this vesicles, this pumps in the hydrogen ion inside this vesicles it becomes acidic and the hydrogen peroxide is formed and that kills the bacteria.

Now, once it kills the bacteria it does not want to keep dead bacteria inside itself it throws it out and then this invagination is gone and it is ready for the next to engulf the rest next bacteria. So, this is the process you know a kind of digressed and I am supposed to teach this any way little later in the course so kind of digressed and went forward.

So, this is a the Cyrokines stimulate immune selectivity and this is how you know how the immune selectivity works. Then there is Interferon, this is again so these are neurotransmitters the first two and the second next two are a ones that stimulates

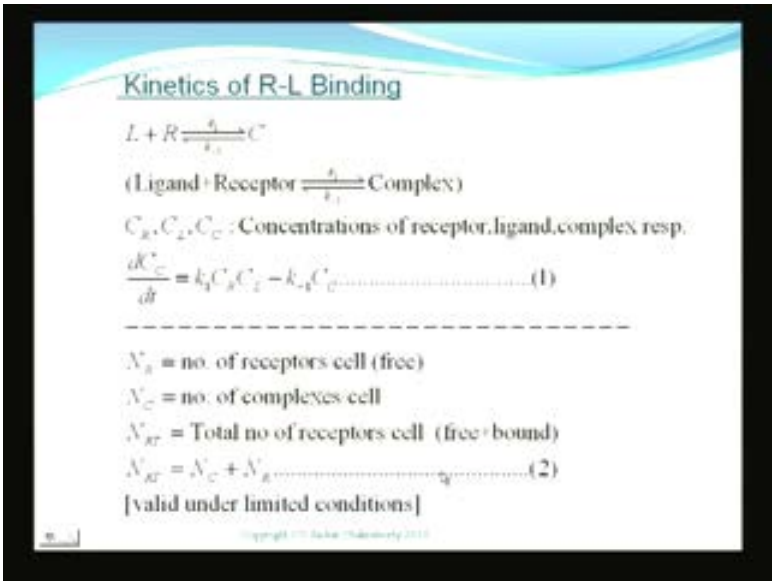
immune activity. So, this is Interferon is also a Cytokine, but it is a one that interferes with the replication of viruses, it is a different you know, it is prevents a viruses from replicating in your system.

Then you have insulin you know you are all aware of insulin. It increases glucose up take in the cell and functions as a growth factor. You are aware of that and then other growth factors, receptors that have intrinsic tyrosine kinase activity which stimulates cell deviation, you know we studied this the whole cell division and the cell growth process earlier and there are these cell growth factors which we did not study at that point of time, which are essentially receptor ligand binding which stimulates the growth process by enhancing metabolism and bit different other ways.

So what I am trying to give you is a sort of a landscape picture of the different types of cell surface receptors that are there , in one block of receptors which in neurotransmitters extremely important for the body is an another block of receptor which are immunes, which have immune activity which are equally important for the body and the third kind of receptor is the one that stimulates growth activity.

So, there all equally important just as you need to grow, you need to protect yourself, you need to feel happy also. So, you know all these three are important.

(Refer Slide Time: 29:47)



So, let us now go to the kinetics of receptor ligand binding and as we said that the receptor ligand binding is a process of given by the second order reaction, forward reaction and the first order backward reaction or reversible system of L plus R giving C and k 1 being k and k minus 1 being the forward and the backward rate constants respectively.

So, if you write the equation it is simply del C and del C C, del t C C here being the concentration of the complex times K 1, C r, C l minus K minus 1 C C. Now, what is going do is, in this system whenever we are doing most of this analysis we are not going to measure things in terms of concentration, but in terms of numbers because these receptors are you know isolated points essentially. It is not a continuous system, the receptors that you, understand what I am trying to say. See, if you are measuring a concentration in a liquid it is a continuous system, where as receptors are isolated points on the cell and we can track the numbers of these receptors by labeling them and looking at the looking at them under microscope. So, we are not going to look at a measure that concentration, but we are going to measure their numbers.

So, if I go back to the screen so you will see here that N r is a number receptors per cell, these are the terminology that we are going to use and you may note it down because this is something that we are going to use consistently, so N r is a number of receptors per cell, free receptors and N C is the number of complexes per a cell and N R T is the number of receptors per cell which is either free or bound. So, either it is in the complex form or in the free receptors form. So, N R T is essentially equals N C plus N r. So, this is written, it is valid under limited conditions. This equation is valid under limited conditions, can any of you sort of into it why you think it is valid under limited condition?

If this shown the receptor limited by the (( )).

All the receptors are ejected.

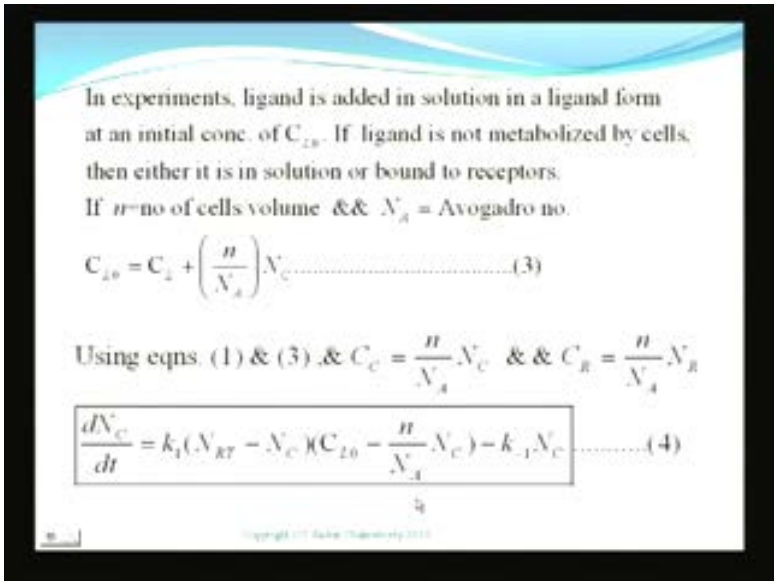
Connected by (( )) by like other ligand.

Yeah, There is several things where that are one of this is correct what you said that the other ligands can bind their receptors, the receptors might die to the more important things actually because even if other ligands are binding I can add in N C 1 plus N C 2

plus N r you know still there is the way to write a balance, but the major problem is receptors and now if it is they do not live forever.

So, they die there is a recycling that is going on and they are again created they are born also. So, those are things that have to be taken into account when we actually do this. So, but in the limited conditions for simple cases we will first assume this is true and then we will look at cases where this is not true also.

(Refer Slide Time: 32:53)



Now, what about the ligands? Now the ligand typically is in the concentration form. The reason the ligand is in the concentration form is because the ligand is in liquid form. So, ligand is measured in terms of concentration where the receptor is measured in terms of number. So, there has to be some conversion that we have to keep doing all the time you know, so in experiments ligand is added in solution in a ligand form or at an initial concentration of C l naught and if the ligands again you can write some sort of a balance equation or some sort of constraint equation for the ligand, but if the ligand is not metabolized by the cell then either it is an solution or bound to receptors. Just like the receptor it is either in the free form or in the bound form similarly, the ligand is either in the solution form or in the bound to receptor form.

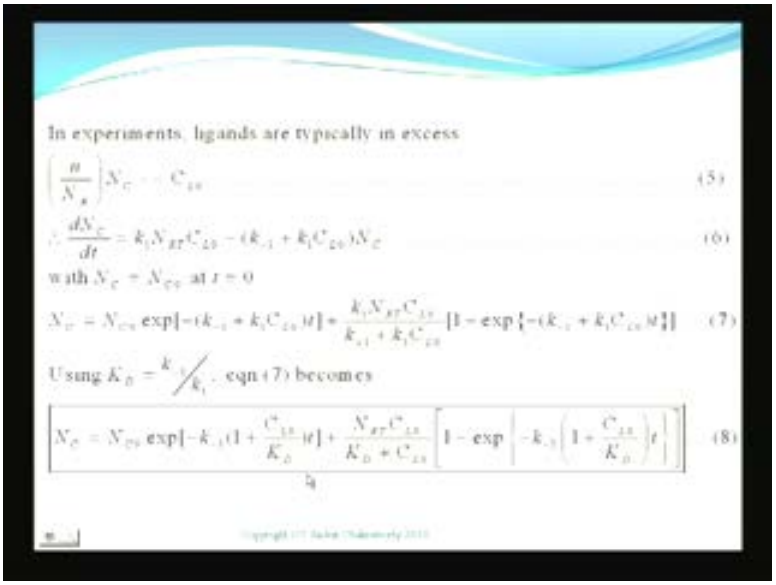
So, if N equals the number of cells per unit volume and N a is avogadro number then N C is a number of just as we defined the number of complexes formed times N over N a

that will give me the concentration of the ligand in the bound form. And C l is a concentration of the ligand in the free form.

So, if you start with the initial concentration of C l naught and we are assuming that ligands are not being added at any point of time after the initial time then C l naught plus C l, C l naught equals C l plus N over N A times N C. Now, what I want to do I had my equation over here.

So, I want to replace this in the in one particular way so if the in a in the terms of N say, so let us replace this here C R and C l everything in terms of N then what you get is C C, it turns out the concentration of the complex is N over N A times N C. Is it correct because just divide multiplied as a number of cell, number of cells per unit volume divided by avogadro number you will get concentration per unit I mean moles per unit volume, so concentration of complex and C R would be N a over N over N a times N R. So, what we do we go back here and replace these concentrations by this so K 1 N, this will be N R T minus N C. Why N R T minus N C from this and this will be here from here C l naught minus N A over N C, C l would be C l naught minus N over N C minus K minus 1 N C. So, equation 4 what it gives us is now I have been able to convert my entire concentration equation into a number equation fine.

(Refer Slide Time: 35:43)



So, now the next assumption that comes in is that N A over N C is much less than C l naught the reason being that you always provide ligands in excess. When you do the

experiments your ligands are not the limiting reactants, is typically the receptors that are they are limiting reactant and that is also physiologically true because what has to bind typically is in excess, typically can always have you know always have occasions or times when the ligand is not in excess, but typically you know for example especially in pathological conditions when there are diseases.

So, ligand may not be in excess, but typically it is a ligand that is in excess and the receptor is not in excess. Ligand that is in excess and the receptor is not in excess, so if you can do that then we can simplify this from equation 4 here, this part we can ignore and we can simplify it a little bit and so N R T this also we can ignore and this N C you can take into here and this part then this part over here you can ignore.

If you look here why we one of the reasons mathematically it helps us to do is solve the equation itself because if you look here it is, I think it is still could be solved then you can try solving it because you can you still or use partial fractions and solve this, to my mind you can still solve this, using partial fractions you just break open the bracket and regroup them as separate you know separate fractions and you can solve them, but here for example, it is much easier and you can do an expand this is a more or less a valid assumption you can do exponential, you can get an exponential solution out of it straight away.

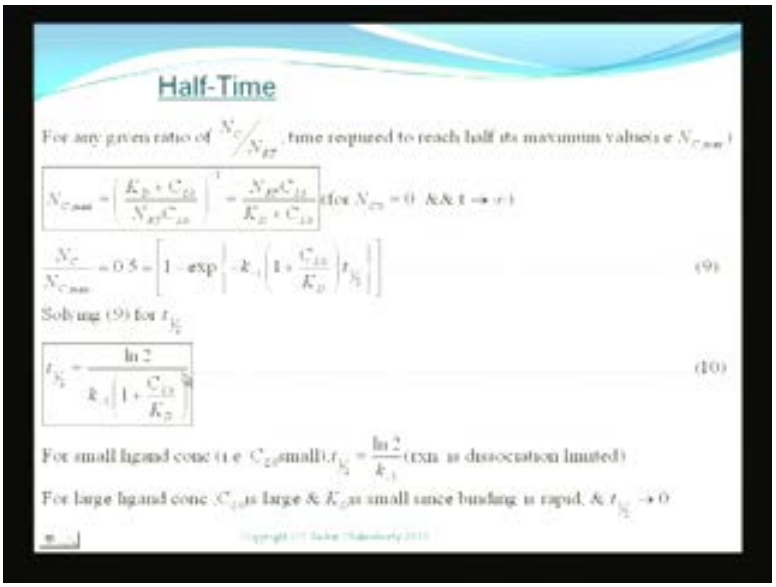
So, if I write my equilibrium constant as K minus 1 over K 1 when this can be slightly simplified as this. Now, can you look at the equation eight over here on the screen and just tell me how it how it is going to look like if I plot my N C the number of complexes that formed with time. So, N C naught is a initial number of complexes present in the system. Let us for the sake of simplicity assume that it is 0 you know it is did not start with any complex at all so how is it going to look now.

Starting with 0 (( )).

I mean that is as simple as that if you do not have the first part, if you have the first part then there is an added time part to that and otherwise it is just saturating out with time you know it is a exponentially growing and saturating out with time because the t going to very large time, this has to saturate to this value N R T, C l naught over K b plus C l naught. And if you have the initial part that is N C naught is not 0 then you have another

exponent out this you can draw the two exponents and then add them up with summation of the two exponents. I will show you the picture in a minute.

(Refer Slide Time: 39:20)



So, from this we come up with the concept of half time. The concept of half time and this just like a half time in straight logical d k and for the weights defined is that of for a given ratio of N C over N R T time required to reach its half its maximum value. So, the maximum value for the complex that is going to be formed half of that, 1 is half the maximum value attained, that is my half time. So N C max out here you can see, if N C naught is 0 let us assume for the case of simplicity then N C max is simply this, so whatever a time, time going to infinity if this is not 0 then it will be N C naught plus this.

So, you know your N C max so all you need to do is half of this so N C over N C max equals half. So N C max is a well known over here should equal this. Is that clear? Why because this is N C max, I can write at C if you ignore this part if N C naught is 0 you can ignore this part and N C could be written as N C max which is this thing over here times this. So for half time you just need to write N C over N C max equals 1 minus exponential this thing on the parenthesis curly brackets equal 0.5.

So, this is what we do and you can solve for your half time and you get comes up similar that what you get in little logical d K. So, half time is l N 2 over k m minus 1 time so 1 over k l naught plus K D. Now, for small ligand concentration if C l naught is small then t half equals l N 2 over k minus 1 this does not make sense you all not doing, but does

not make sense, it does? Let me go to this screen and show you the first thing first one for small.

First one is.

First one we are seeing (( )).

No zeroth order for small ligand concentration t half is given as l N 2 over k minus 1 is that does it make sense? Yes? No, it does not make sense it is the oxymoronic. What did we start with?

Started with the ligand concentration it is not limiting.

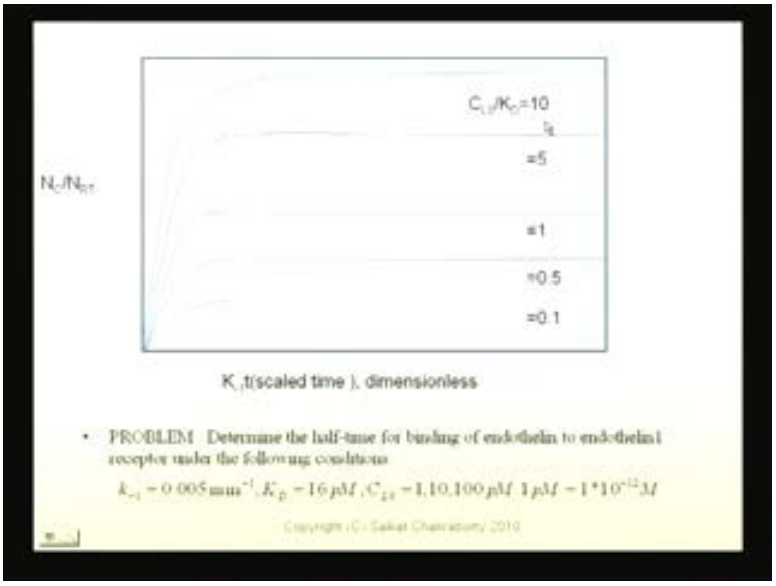
It is.

Not limiting.

It is not limiting, it is very high and then we can cannot assume that C l naught is very small because as soon as you make it very small it also becomes small as compared to N C the concentration of the complex. So, this is though it is theoretically it makes sense, but it is oxymoronic in general because you cannot assume it to be first a very large concentration and then make assumptions and simplifications based on that factors very small. The second of course, makes sense that is C l naught is very large and K D is very small then this term is very high and then t half will go to 0 which means that within a very short period of time, the half time would be reached and the N C max, half of N C max would be reached within a very short period of time, but that remember has you to do with not just k l not very large, but also K D very small. K D being the, what is K D? K D is the dissociation rate constant so here the back the backward over the forward rate constant.

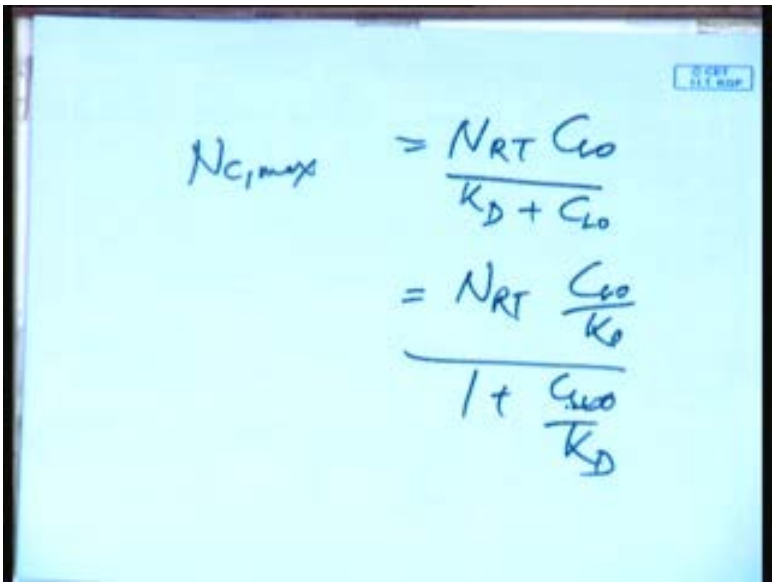
So, K D being very small means that the forward rate constant is much higher than the backward rate constant which means that not just that the ligand is present in the large amount, but the reaction is taking place in the forward direction very fast. So, these combine effects of these two facts that the ligand is present in large amount and the reaction is taking place in the forward direction much faster than in the backward direction will make my half time very small, it is physically meaningful.

(Refer Slide Time: 43:24)



Now, let me show you this plot. So, this how it looks like if I had if my N C initial N C is 0 this is what it is you know N C naught equals 0, then this is how it looks like. So, if you see k C l, it depends on C l naught over K D and these are various values so it saturates out to a value if I look at, it will saturate out to this value.

(Refer Slide Time: 43:55)



And this one, N C max you can write that as N R T times N C max I can equals N R T, C l naught over K D plus C l naught and by divide all through by say C l naught I get C l naught over K D plus 1 plus C l naught over K D. So, that is what we are drawing in the

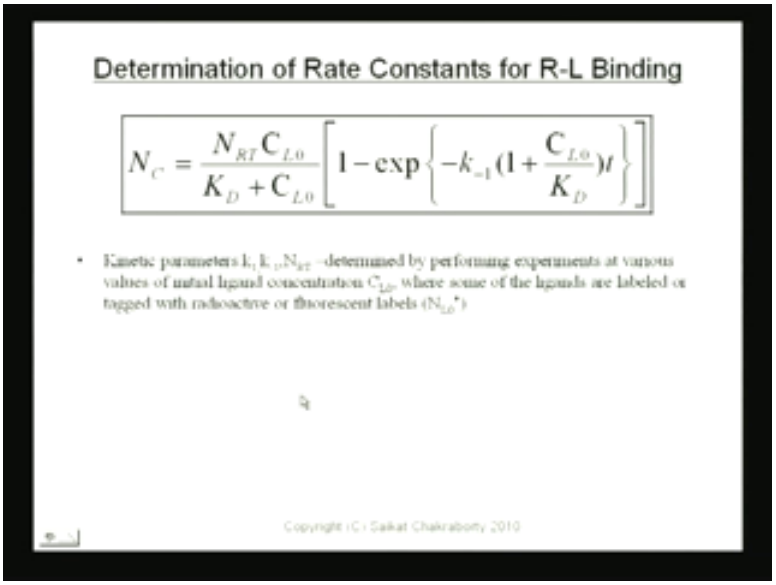
plot over here. So, C l naught over K D is a single parameter so I have been able to convert this 2 parameter into a single parameter system now and as it changes this you know it increases now this, what does this remind you of now?

Michaelis menten (( )).

This reminds us of the Michaelis Menten kinetics. This is exactly you know the way I wrote it over here if you look here. So, C l naught this is exactly looks like the Michaelis Menten kinetics. So, then there is let us, so I want you why do not you write down this problem, I want you to write down this problem and then I will give you another problem, what I want you do is that do these problems on a sheet of paper and submit it in the next class next week as an assignment with a name and roll number written this under be another one, I will give at the end of the class.

So, determine the half life for binding of endothelin to endothelin 1 receptor under the following conditions. Determine the half time for binding of endothelin to endothelin 1 receptor under the following conditions. K minus 1 equals 0.005 minute inverse K D equals 16 picomolar C l naught equals 110, so these are different values you know I want you to do for these different values. 1, 10 and 100 picomolar and 1 picomolar is defined as 1 into 10 to the minus 12 molar, finished.

(Refer Slide Time: 46:30)



Let us go further now, what the next thing that we want to do is the determination of the rate constants of the receptor ligand binding. You want something I will repeat one more time, determine the half time for binding of endothelin to endothelin one receptor under the following condition K minus 1 equal 0.005 minute inverse K D 16 picomolar C l naught is 110 and 100 picomolar for 3 different value, 1 picomolar being 1 into 10 to the minus 12 molar.

So, the next thing that we need to do is some of these constants we need to evaluate because otherwise there is no way forward, how do we evaluate these constants and then later on during the course we will try and understand how we evaluate these constants experimentally not just theoretically. So now let us talk about how to just you do some plots and evaluate these constants. So my expression the kinetic equation is given like this so in this is a case where N C naught equals 0.

So, it is given as N R T C l not over K D plus C l naught times 1 minus exponential this factor out here. Now, the kinetic parameters here are the forward and the backward rate constants K 1 and K minus 1 because you know you have the dissociation constant and the backward rate constant coming out separately. So, you have 2, there are 2 constants there, there is no running away from that. N R T being the total number of receptors that is another one and we need to perform a experiments at various values of the, so one of the result that talked talk I would not talk about the experimental procedure, I will talk about that later in the course, but right now theoretically if you look at it you know without thinking as a experimental procedure theoretically one of the ways is to perform these experiments just like as you do. This is like a first order system and you know as you do in all kinds of us. Why is this a first order system by the way? Is this a first order system to start with? Is this a first order system or not?

Yes

Yes it is, why is it first order system?

(( )) first order ratio

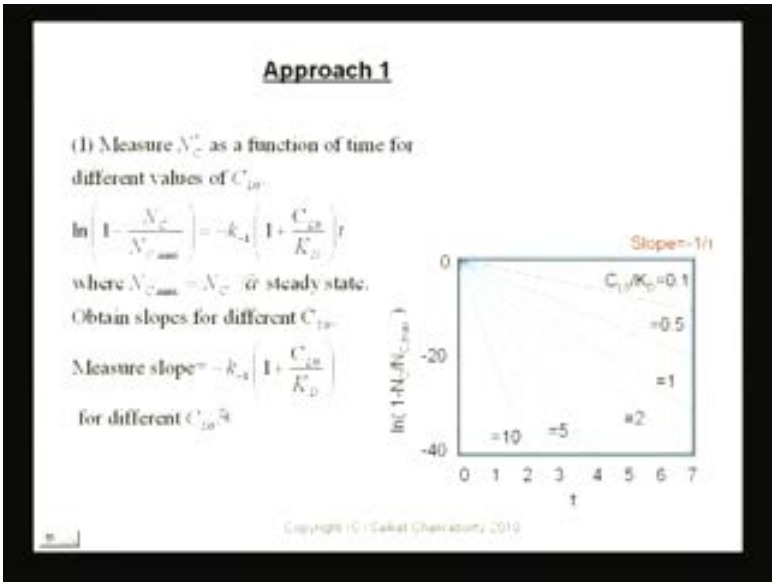
We go (( )) why because of assumption.

(( ))

What is that because of the assumption we made that the C l the ligand this present in large amount otherwise it is second order in the forward direction. So, when we study the kinetics in the system it is simply as simple as the kinetics study of the that we did for a first order six system, so you perform experiments at various values of the C l naught the ligand concentration and then as I told you as I showed you just before at the beginning is that essentially how do you do that is you tag the ligands. The ligands that you want to or if they are not tagged you cannot measure, you tag them with a fluorescents, you know make them fluorescent so that they will glow and you they that is a way you measure them.

So, this is a formula and if you do not think about all the details of the process which will deal with later you essentially are carrying out your experiment if you look at your problem last problem, what we did was C l naught we carry the different values of C l naught, we carried out the whole experiment at different values of C l naught. So, here also you carry out the experiment at different values of C l naught and you measure the amount of constants, amount of complexes that is formed because these are all tags so these are fluorescent so because they tag you can measure them in the tag form.

(Refer Slide Time: 49:48)



So, you measure N C as a function of time for different values of C l naught, you start we know how much C l naught you are putting in and you measure your N C because it is tagged and you know so it will emit fluorescence. We measure them with time, as a

function of time for different values of C l naught and as I showed you last time also I just here that C l naught over K D is a parameter you know you can divide the whole equation by K D.

So, C l naught over K D would be a parameter running parameter and as you keep increasing your C l naught over K D the slope would be changing because as you keep increasing your C l naught over K D what does it mean that the reaction should proceed in the forward direction more because you have increased the amount of ligand that is present and you have decreased increased the forward rate constants.

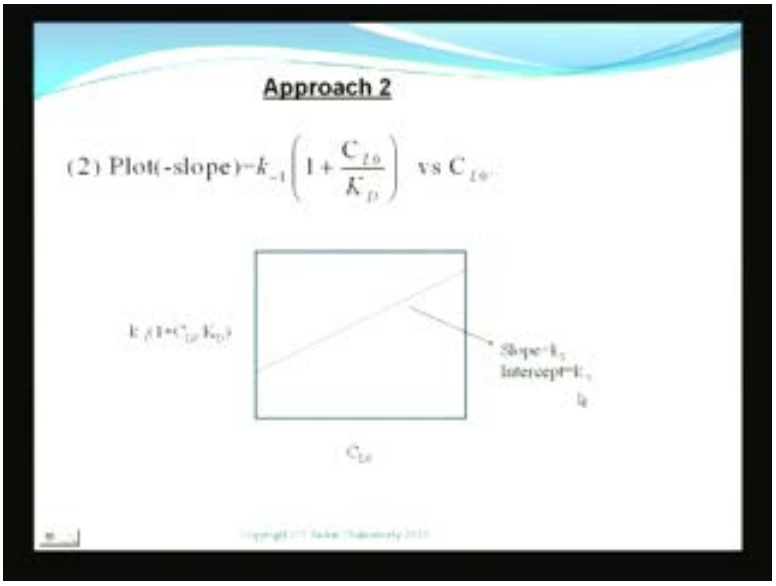
So, I will just go little quickly because I need to go I want to go to the problem. So, this is I am, so the slope here let us let us be a little quick. So if you take the log of this you know so 1 minus N C over and N C max on this side, log of this here so this is my N C max by the way. So, N C over N C max you can takes 1 N C minus N C N C over N C max is 1 minus exponential of this term. which means that 1 minus N C over N C max is simply the exponential. Now, you can take log of both sides so you will get l N 1 minus N C over N C max equals minus K 1, 1 plus C l naught over K D is that correct? These are times t. Now, at steady state you know N C equals N C max steady means, what does steady state mean?

Infinite time.

Infinite time, t going to infinity in this case, this is the only steady possible no other steady state. So, the slope here is simply this if I plot this 1 minus N C over N C max, I know how much my N C max is going to be because I know how much ligand I gave in and so if I plot this 1 minus N C over N C max.

So I am, once it attains steady state I can measure my N C max. If I plot this with time then this is my slope and from this slope I can I C l naught is known and for different values of slope I can get these constants because so with C l naught you know so this is 1.

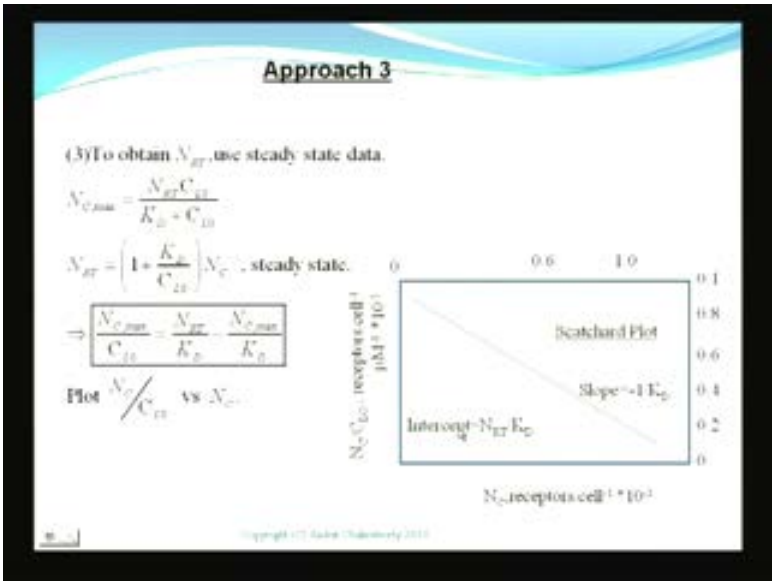
(Refer Slide Time: 52:04)



So, you measure this slope for different values of C l naught next step is this one. The plots the slope once you calculated with slope for different values of C l naught, plots the slope for different values of C l naught and then from this you can calculate the intercept from this intercept and the slope of this you can calculate both the constants is that clear.

So, there are 2 unknowns out here in the slope, but what you do is you now again plot the slope with C l naught and from the intercept and the slope of this, so the intercept will give you just K minus 1 I believe and the slope will give you K 1 or something like that. So, slope will give you K 1 and intercept will directly give you K minus 1. So, then you get both constants.

(Refer Slide Time: 52:50)



So, this is 1 approach and the other approach very quickly let me do may be and if I need to I will come back next class. So, to obtain N R T use N C max equals and N R T times C l naught K D plus C l naught. Now, N C max is something that you know and then you can back calculate and find your N R T and this is the plot that you do and I will come back to this later on, the plot that you do to do this. So, essentially want get your N R T and this will intercept of this is known as Scatchard plot. This is very important plot and we will come back to it again and again this known as Scatchard plot. N C over C l naught you might want to make a quick note or at least of the axis.

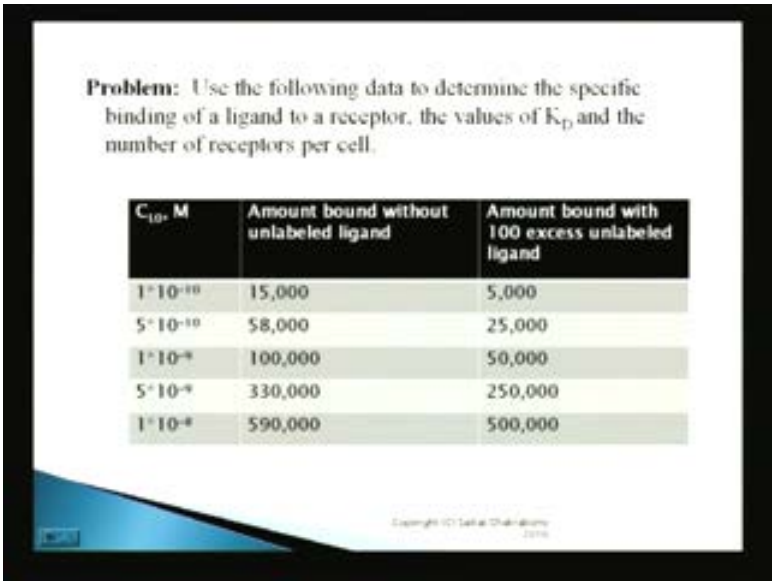
So, the way you do it is you make a Scatchard plot of N C over C l naught and this is receptors per cell, cell inverse that is a unit of this and over N C. And Scatchard plot is what kind of plot is it a steady state, unsteady state what kind of plot? What kind of plot is Scatchard plot? Steady state plot.

So, essentially this is what you are plotting. This box out there. So, N C max over C l naught though it is written I have written here N C, but it is actually N C max that you are plotting and N C max means that steady state has been attained. So, N C max over C l naught equals N R T over K D minus N C max over K D. So, if you plot this over this then what you get you get your intercept is this and slope is 1 over K D or minus 1 over K D, so you can directly calculate your N R T.

So, in a way as I said that if know your K D, if you like you know you already know your C l naught, if know your forward and the backward rate constant you can know your K D so you can back calculate from N C max to calculate your N R T and this is process. The reason we assume this process is because we know do not need 1 data, we want to use multiple data point to minimize error.

So, this is the way. So, Scatchard plot remember most important thing to remember, it is a steady state plot. What you plot essential this steady state value of N C over C l naught versus C l naught, the intercept will give you N R T over K D is that slope will give you K 1 minus 1 over K D.

(Refer Slide Time: 55:07)



So, the last thing I want you to write is this problem. So, these 2 problems you are going to do together and submit as an assignment in the next class. So, the problem is use the following data to determine the specific binding of a ligand to a receptor. The values of K D and the number of receptors per cell.

So, basically you need to find all the rate constants and N R T and C l naught is given in molar 1 10 to the minus 10, 5 10 to the minus 10,1 10 to the minus 9, 5 10 to the minus 9 and 1 10 to the minus 8. So first case is, when there are no unlabeled ligands are there in the system and second case is, when there are 100 excess unlabeled ligand and there is a data that is given to you. So, 100 excess unlabeled ligands per cell by the way. And these

are N C values that are thousands, are numbers that are given in thousands are the N C value you obtain. N C values are steady state or in other word the N C max.

So, the hint is obviously you have to use something like a Scatchard plot to do this because this is a steady state values that are given and, but there are some tricks involved in it and some. ((no audio 57:13 to 57:55)) I hope you have already written down. So, we can stop here and we will continue in the next class. Thanks.