

Fundamentals of Protein Chemistry
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Module - 05
Protein ligand interactions
Lecture - 22
Kinetics and Thermodynamics of Protein - Ligand Binding

We continue our discussion on protein ligand interactions. In this lecture we will be looking at the kinetics and the thermodynamics of the protein ligand binding process.

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CONCEPTS COVERED

- Binding of ligands to a macromolecule
- Protein ligand binding kinetics ✓
- Thermodynamics of protein-ligand interactions ✓
- Protein-ligand binding models

The slide features a video inset of Prof. Swagata Dasgupta in the bottom right corner. At the bottom, there are logos for IIT Kharagpur and NPTEL.

For this particular lecture, we will be looking at binding of ligands to a macromolecule, the protein ligand binding kinetics, the thermodynamics of the protein ligand interactions and specific binding models. We had looked at some of these in the previous lecture, where we saw the specifics of binding and how this occurs and few examples of the specificity and the affinity.

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KEYWORDS

- Dissociation constant
- Cooperativity
- Scatchard plot

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In this lecture we will be looking at concepts like, the dissociation constant and the Scatchard plot, which is a common plot in protein ligand binding.

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- Biological systems can be usually described as having constant pressure P and constant temperature T
 - the system is free to exchange heat with the surrounding to remain at a constant temperature
 - it can expand or contract in volume to remain at atmospheric pressure

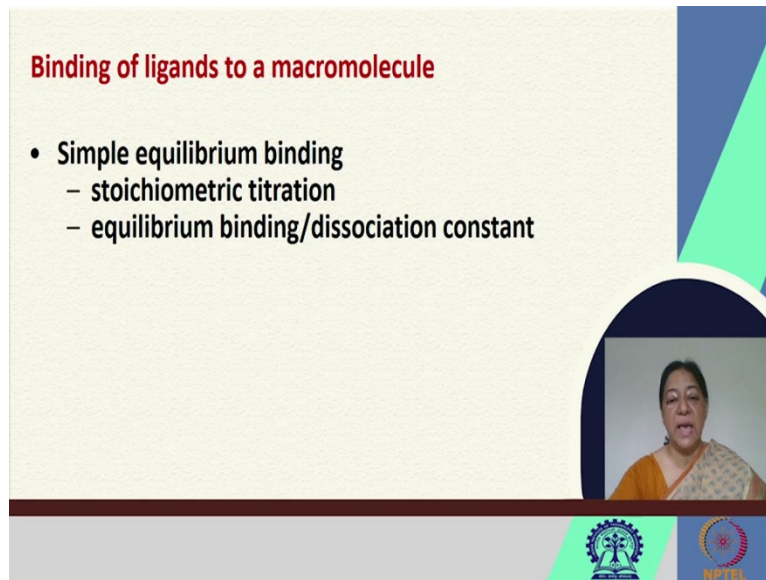
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The biological systems that we consider, can be described as having constant pressure and constant temperature. The system is actually free to exchange heat with the surroundings, to remain at a constant temperature and it can expand or contract to achieve this constant temperature.

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Binding of ligands to a macromolecule

- **Simple equilibrium binding**
 - stoichiometric titration
 - equilibrium binding/dissociation constant

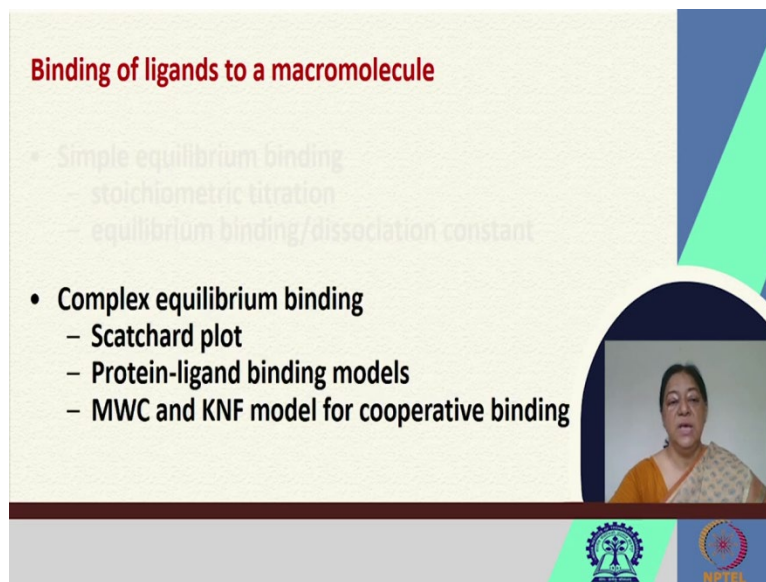


If we look at the bindings of the ligands to the macromolecules, there is simple equilibrium binding, where we look at a stoichiometric titration and other concepts of equilibrium binding and dissociation constants.

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Binding of ligands to a macromolecule

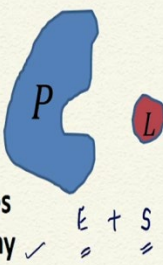
- Simple equilibrium binding
 - stoichiometric titration
 - equilibrium binding/dissociation constant
- **Complex equilibrium binding**
 - Scatchard plot
 - Protein-ligand binding models
 - MWC and KNF model for cooperative binding





In addition to this, there is complex binding, where we may have a larger number of binding sites of a particular ligand to the macromolecule.

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Macromolecule ligand binding



Biologically relevant macromolecules such as proteins are involved in many interactions with other molecules that include other proteins, nucleic acids, membranes, small molecule ligands, and the surrounding solvent molecules.

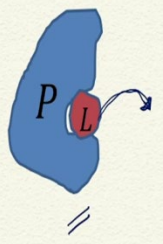



Looking at biological relevant macromolecules such as proteins. They are involved as we looked at in the previous lecture in many interactions with other molecules. These molecules could include proteins, nucleic acids, membranes, small molecule ligands and even the solvent molecules.

So we have our protein of interest and we have our ligand molecule. The enzyme substrate example is a unique example of this substrate forming an enzyme substrate complex, which is a subset of our protein ligand binding.



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Macromolecule ligand binding



$$P + L \rightleftharpoons PL$$

$$K_A = \frac{[PL]}{[P][L]}$$

$$K_D = \frac{[P][L]}{[PL]} = \left(\frac{1}{K_A}\right) \therefore [PL] = K_A [P][L]$$



If we look at the protein and we understand that this ligand is now bound in the active site, by the law of mass action, we can say that we have in the particular stoichiometry, (where we consider a 1 is to 1 stoichiometry) the protein and the ligand interact to form the protein ligand complex.

We have to remember that this is an equilibrium situation, where we may have the ligand unbound from the protein ligand complex. So this would result in an association and the reverse process would be a dissociation. When we look at the association constant the K_A value, we have

$$K_A = \frac{[PL]}{[P][L]}$$

When we consider the dissociation constant, since it is the reverse of this reaction, we realize that this is going to be the inverse of the association constant. This is important in an understanding of how strong the complex is. For example, if we want an enzyme substrate complex to form, we would not want the binding to be too tight, because then the substrate would not be released from the ligand or the product bound to the active site would not be released.

However when we are designing an inhibitor, in that case we would want this complex to be really tight, having a very high affinity and having a very tight association. So when we are looking at the concentration of the protein ligand, we see that this is a product of the association constant, the protein and the ligand concentrations.

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Intermolecular association can be described by two rate constants

$$P + L \xrightleftharpoons[k_{off}]{k_{on}} PL$$

$$P + L \xrightleftharpoons[k_{-1}]{k_1} PL$$

$$v_{forward} = k_{on}[P][L]$$

$$v_{reverse} = k_{off}[PL]$$

Units of $v_{forward}$ and $v_{reverse}$ are $M s^{-1}$ ✓
 Units of k_{on} are $M^{-1} s^{-1}$ ✓
 Units of k_{off} are s^{-1} ✓

The intermolecular association can actually be described by two rate constants. So, if we look at the forward reaction we have a rate constant that is called k_{on} . We have the reverse that is the k_{off} , saying that the ligand is on or off the active site. We can call this the k_1 and the k_{-1} , these being small 'k's indicating rates.

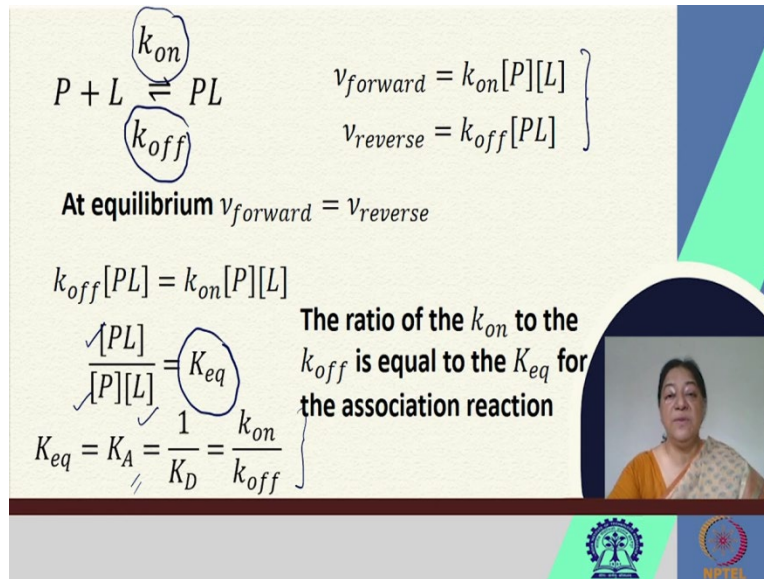
Now if we look at the forward reaction, then we have

$$v_{forward} = k_{on}[P][L] \quad \text{and}$$

$$v_{reverse} = k_{off}[PL]$$

If we look at the units of the forward and the reverse reactions, we realize that these are given by concentrations into second inverse. And the concentration of k_{on} are $M^{-1}s^{-1}$ and s^{-1} of k_{off} .

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Now that we have our equilibrium situation described by actually two rate constants, we have to reach an equilibrium where the forward rate and the reverse rate are the same. To do that we have to have an equality in terms of the forward and the reverse rate, given by these expressions here [refer to slide]. And we then have an equilibrium concentration of the protein ligand complex, the protein and the ligand.

This equilibrium concentration gives us our association constant, the inverse of it - the dissociation constant, which is the ratio of the k_{on} and the k_{off} . The ratio of the k_{on} to the k_{off} is therefore, equal to the equilibrium constant for the association reaction. So, we have to remember that when we are determining the thermodynamics of a protein ligand binding system, we have to determine the association constant, we have to ensure that the reaction has reached an equilibrium.

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$$\frac{[PL]}{[P][L]} = K_{eq} = K_A = \frac{1}{K_D} = \frac{k_{on}}{k_{off}} \checkmark$$

- For tight binding $K_{eq} \gg 1$, $K_D \ll 1$
- k_{on} is very large ($10^8 \text{ M}^{-1} \text{ s}^{-1}$) ✓
- k_{off} must be very small ($10^{-2} - 10^{-4} \text{ s}^{-1}$) ✓
- It can be shown mathematically that the rate at which two simple object associate depends on their radius and effective molecular weight.




Now that we have an understanding of what the association constant is; the inverse of the dissociation constant, it is a ratio of the k_{on} , k_{off} values. For tight binding we realize, that we would want the K_A value to be very high, much greater than 1, which would indicate that we would want a K_D value to be very low.

So a low K_D value is indicative of tight binding. This means that if k_{on} is very large, then k_{off} must be very small. This can be shown mathematically, that the rate at which the two simple objects associate, would depend upon their radius and their effective molecular weight and even the orientation in which the molecules come together to form the protein ligand complex.

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- Let us assume that the rate at which P and L associate is diffusion limited. ✓
- The theoretical k_{on} is about $10^8 \text{ M}^{-1} \text{ s}^{-1}$. Therefore, if K_D is known, the first order rate constant for dissociation, k_{off} can be estimated
- k_{off} can also be determined experimentally.

Diffusion limited or diffusion controlled reactions are those reactions where the reaction rate is equal to the rate of transport of the reactants through the reaction medium (solution). ✓

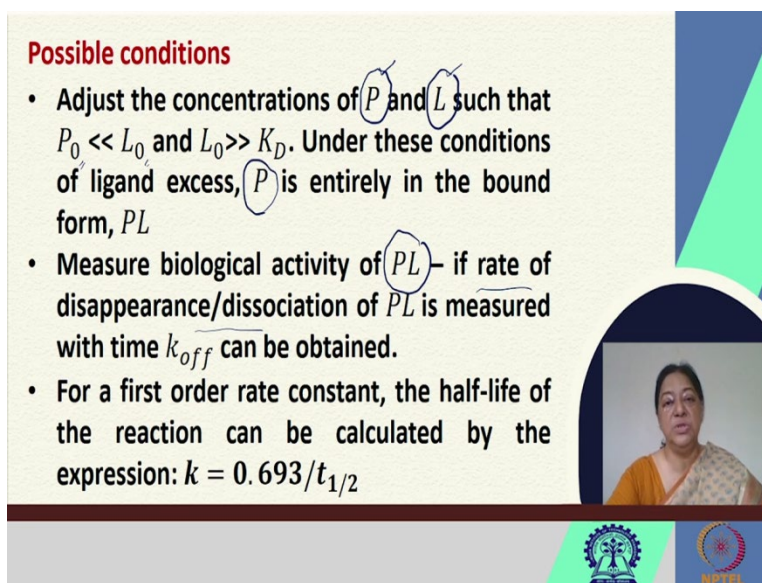




If we assume that the rate at which P and L associate is diffusion limited or the diffusion controlled reaction, where the reaction rate is equal to the rate of the transport of the reactants

through the reaction medium, as there is a limit by which we can have the motion of the molecules.

The theoretical value for k_{on} is around $10^8 \text{ M}^{-1}\text{s}^{-1}$. So if K_D is known, we can determine what k_{off} is, because we know that the association constant K_A , being the inverse of K_B , is equal to k_{on} by k_{off} .

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Possible conditions

- Adjust the concentrations of P and L such that $P_0 \ll L_0$ and $L_0 \gg K_D$. Under these conditions of ligand excess, P is entirely in the bound form, PL
- Measure biological activity of PL – if rate of disappearance/dissociation of PL is measured with time k_{off} can be obtained.
- For a first order rate constant, the half-life of the reaction can be calculated by the expression: $k = 0.693/t_{1/2}$

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Now the possible conditions that we can look at is, as we are designing the experiment we can adjust the concentrations of the protein and the ligand. The 0 substrate in each case means, the initial concentrations that we have started off with. In the conditions of ligand in excess, we can assume that the total protein that has been taken is bound by the ligand, because we have taken the ligand in excess.

We can measure the biological activity of PL that is the complex, the rate of disappearance or dissociation of the PL and the k_{off} . This is dependent on what we can observe. For a first order rate constant we know that the half life of the reaction can also be calculated.

As one who is going to design an experiment, we start off with specific concentrations of protein and ligand. We allow the situation to reach an equilibrium, which means that the concentration of PL will increase; we saw such an example in the previous lecture, which we will revisit in our experimental observations of protein ligand binding.

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Macromolecule ligand binding

Let v represent the moles of bound ligand per mole of protein:

$$v = \frac{[PL]}{[P]_{total}} = \frac{[PL]}{[P] + [PL]}$$

$[P]$ and $[L]$ represent *unbound* protein and ligand respectively.

$[P]_{total}$ and $[L]_{total}$ represent the total amount of protein and ligand in the system



When we look at macromolecule ligand binding, we can describe a quantity v that represents the moles of bound ligand per mole of bound protein. So we have:

$$v = \frac{[PL]}{[P]_{total}} = \frac{[PL]}{[P] + [PL]}$$

This means, that we have an amount of free protein and the rest of the protein is bound with the ligand.

So this [refer to slide] would be the moles of bound ligand and this would be the amount of protein that we have, which would either be in the bound form or in the unbound form. The P and the L correspond to the unbound protein and ligand and $[P]$, $[L]$, $[P]_{total}$ and $[L]_{total}$ represent the total amount of protein and ligand that we have in the system.

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$$v = \frac{[L]}{K_D + [L]}$$

[L] is the concentration of unbound ligand, which is not the same as $[L]_{total}$.


Certain binding experiments involve measuring the concentration of bound ligand ($[PL]$) instead.

In this case, you can easily calculate [L]:

$$[L] = [L]_{total} - [PL]$$

It may also be that $[L] \approx [L]_{total}$

This is true when $[L] \gg [P]$.



What we can do is from the previous expression that we just looked at, we can rearrange this to work it to form v being given this [refer to slide] expression, where L is the concentration of the unbound ligand, which is not the same as $[L]_{total}$. And certain binding experiments as we just looked at, measure the concentration of the bound ligand and from that we can actually calculate the free ligand.

Depending upon how we have designed the experiment, if the total ligand concentration is very high, then we can assume that the free ligand is approximately equal to the total ligand. We will look at these expressions for the amount of bound ligand per macromolecule in the subsequent slides.


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Macromolecule ligand binding

$$v = \frac{[PL]}{[P]_{total}} = \frac{[PL]}{[P] + [PL]}$$

$$v = \frac{[PL]}{[P] + [PL]} = \frac{K_A [P][L]}{[P] + K_A [P][L]} = \frac{K_A [L]}{1 + K_A [L]} = \frac{[L]}{K_D + [L]}$$

In many experimental procedures v can be measured at a range of ligand concentrations from which K_D can be estimated and then $\Delta G^0_{binding}$



So, we are looking at: $v = [PL] / [P]_{\text{total}}$ that is the protein ligand concentration of the complex divided by the $[P]_{\text{total}}$ which we found was the unbound protein and the bound protein. So if we look at this expression we found out from one of the expressions earlier, with the knowledge of what we mean by K_A , we can find out the value or we can express our new value in terms of just the free ligand concentration.

In many experimental procedures v can be measured at a range of ligand concentrations from which we can find out the value of the dissociation constant followed by the free energy of binding.

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K_D values in biological systems

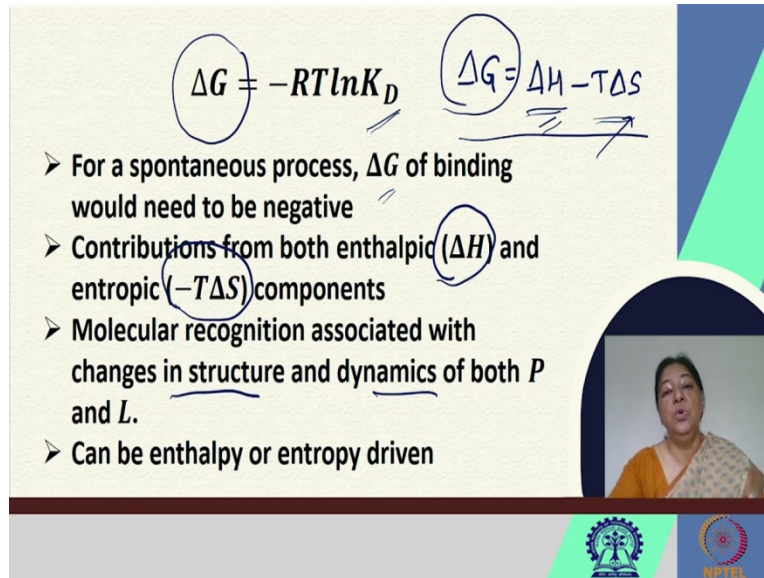
- Monovalent ions binding to proteins or DNA
 K_D 0.1 mM to 10 mM
- Allosteric activators of enzymes e. g. NAD
 K_D 0.1 μ M to 0.1 mM
- Site specific binding to DNA
 K_D 1 nM to 1 pM
- Trypsin inhibitor to pancreatic trypsin protease
 K_D 0.01 pM
- Antibody-antigen interaction
 K_D 0.1 mM to 0.0001 pM

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If we look at some specific K_D values for biological systems, here [refer to slide] are some examples where we see monovalent ions binding to proteins or DNA; they are in the mM range. When we look at activators of enzymes, allosteric activators, they range from μ M to mM range.



When we have site specific binding to DNA, they go even better as nM to pM. Trypsin inhibitor binding to the protease is also in the pM range. And we can have antibody-antigen interaction that can go to a very high specificity, very high affinity, with very low values for the dissociation constants, meaning that these interactions are very tight.

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$\Delta G = -RT \ln K_D$ $\Delta G = \Delta H - T\Delta S$

- For a spontaneous process, ΔG of binding would need to be negative
- Contributions from both enthalpic (ΔH) and entropic ($-T\Delta S$) components
- Molecular recognition associated with changes in structure and dynamics of both *P* and *L*.
- Can be enthalpy or entropy driven



If we look at our value now of the dissociation constant, we can look at a ΔG , the free energy of binding. Now, we know from our expression of ΔG , that ΔG is equal to ΔH , $-T\Delta S$. This implies that we have enthalpic and entropic components in our ΔG of binding. Molecular recognition is associated with changes in structure and dynamics of both the protein and the ligand.

We have to realize that there are associations in terms of the electrostatics, the hydrogen bonding, the hydrophobic interactions, Van der Waals interactions, that will contribute to the enthalpy of this overall process. Again we are looking at an association, so there will be an entropic contribution, not only from the association of the two molecules coming together, but also by the removal of the water molecules from the surrounding or from the environment of the active site of the macromolecule and from the surface of the ligand. This will all contribute to the enthalpic and the entropic contributions that would give us an idea of what ΔG is. But we know that for the spontaneous binding, we would need to have a ΔG that is negative.

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Macromolecule ligand binding

- Interactions involve a high degree of specificity and affinity
- Require an understanding of the forces to obtain a description of the thermodynamics of binding
- To correlate thermodynamic parameters with the structures of the macromolecule and ligand

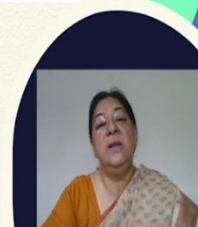
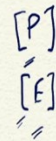
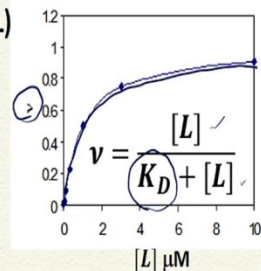


The interactions of macromolecules and ligands, involve a very high degree of specificity and affinity, an understanding of the forces so that we can get a correct description of the thermodynamics of binding.

And based on this, we can actually correlate the thermodynamic parameters with the structures of the macromolecules and the ligands. In trying to understand what are the components present in the active site of the protein or what are the specific moieties the chemical description of the ligand that is going to result in a favorable interaction for the macro molecular ligand binding.

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- K_D is a concentration (mol/L)
- K_D gives the concentration of ligand that saturates 50% of the sites



If we get a further understanding of what the dissociation constant actually means and now [refer to slide] look at the v versus the L . When we saw the specific expression for v versus L , we saw

that we can look at a ligand concentration and we have a K_D value here. If we look at the K_D this actually gives us the concentration of the ligand that saturates 50% of the sites.

We have to realize that we get this saturation, because the macromolecule has a limited number of binding sites. So there is a saturation that will be absorbed in any of these protein ligand experiments, as well as enzyme substrate experiments. Because there is a specific concentration of protein in this enzyme; we have a definite concentration of the protein and the enzyme.

So, there are a limited number of active sites to which the ligand can bind.

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➤ K_D is a concentration (mol/L)
 ➤ K_D gives the concentration of ligand that saturates 50% of the sites
 ➤ Almost all binding sites are saturated if the ligand concentration is $10 \times K_D$
 ➤ $\Delta G = -RT \ln K_D$

$$v = \frac{[L]}{K_D + [L]}$$

$$K_D = \frac{[P][L]}{[PL]} = \frac{1}{K_A}$$

When we look at this specific interaction, we see that $K_D = [P][L] / [PL]$; that can also give us the value of the association constant. Now, almost all the binding sites are saturated usually, if the ligand concentration is 10 times the K_D value. So we want to look at the value of 0.5 here that corresponds to our K_D value.

And from the K_D value, as we looked at previously, we can determine the free energy of binding.

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Macromolecule ligand binding

$$v = \frac{[PL]}{[P]_{total}} = \frac{[PL]}{[P] + [PL]}$$

$$v = \frac{[PL]}{[P] + [PL]} = \frac{K_A [P][L]}{[P] + K_A [P][L]} = \frac{K_A [L]}{1 + K_A [L]}$$

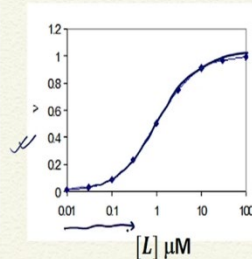
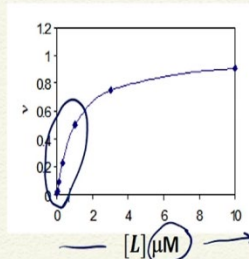
In many experimental procedures v can be measured at a range of ligand concentrations from which K_A / K_D can be estimated



So we have our new value, we have our expression. Now in many experimental procedures we can measure v for a series of ligand concentrations, as we looked at and from that we can determine either the K value or the K_D value, as we saw from a specific plot of the v versus L .

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By using a logarithmic scale for the x-axis, as shown below for the same binding data, the data get more spread out at low $[L]$ and form a characteristic S-shaped curve. The best results are obtained if binding data over several orders of magnitude in $[L]$ can be collected.



Now when we look at the v versus L plot, it is difficult to understand the actual number of binding sites or a K_D value. So the best thing would be to linearize the plot. But if we want to go for a larger range of concentration along the x axis as shown here [refer to slide], we go for a logarithmic scale and the advantage of using this is, we get the data that is spread out at a lower ligand concentration, forming this characteristic S curve and the best results are obtained if we can get binding data over several orders of magnitude.

Because if we look at the initial stage here, we will see we have many values for the v , for a smaller span of ligand concentration. So, if we use a logarithmic scale we can actually spread this out into an S shaped curve.


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Scatchard Plots

The binding data can be linearized to give the Scatchard plot

Starting with equation $v = \frac{K_A [L]}{1 + K_A [L]}$ ✓

multiplying both sides by $1 + K_A [L]$ yields:

$$v + v K_A [L] = K_A [L]$$


We have a look now at Scatchard plots, where the binding data can be linearized to give the Scatchard plot that is given by a specific equation. So we start with the equation v , the definition of which we know is the $[PL] / [P]_{\text{total}}$, the total macromolecule. If we multiply both sides of this equation we get this set: $v + v K_A [L] = K_A [L]$

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Scatchard Plots

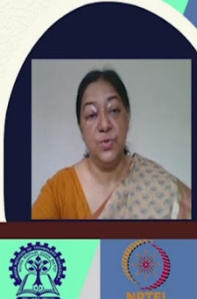
$$v + v K_A [L] = K_A [L]$$

Dividing through by $[L]$ gives $\frac{v}{[L]} + v K_A = K_A$

which rearranges into the following form:

$$\frac{v}{[L]} = K_A - v K_A$$

A plot of $v / [L]$ versus v should give a straight line for a binding reaction with identical sites. The slope of this plot is $-K_A$ and the y-intercept is K_A



Now if we do a bit of algebra, where we have $v + v K_A [L] = K_A [L]$, K_A being our association constant. When we rearrange this equation, we have the following form of the equation. We see that if we can plot $v / [L]$ versus v , we can get a value for the K_A , as the slope of the plot and the y intercept.

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Multiple binding sites - n identical and independent

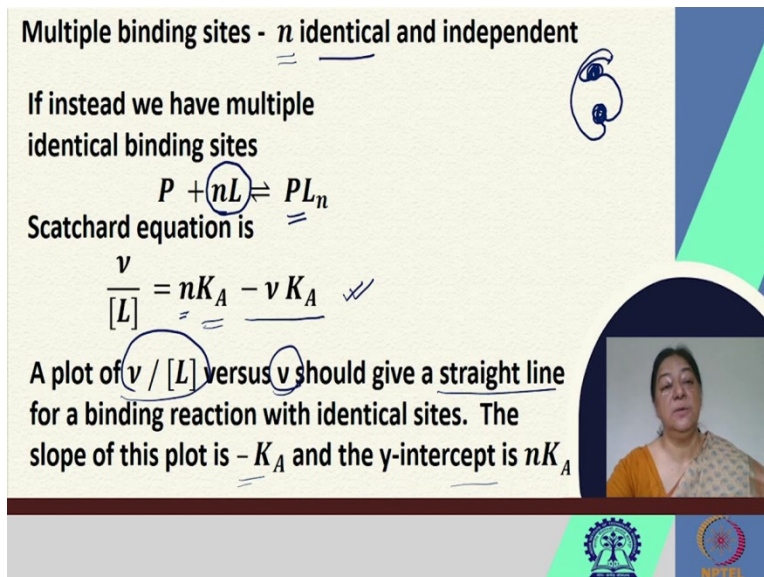
If instead we have multiple identical binding sites

$$P + nL \rightleftharpoons PL_n$$

Scatchard equation is

$$\frac{v}{[L]} = nK_A - vK_A$$

A plot of $v / [L]$ versus v should give a straight line for a binding reaction with identical sites. The slope of this plot is $-K_A$ and the y-intercept is nK_A



This gives us a value of the association constant. It may so happen that we have multiple binding sites on the protein. Where we are looking at [refer to slide] specific and active site specific here or we can have a different binding site here, where we could have a ligand molecule here, have a ligand molecule at this position also. Which means that, we have to account for possibilities of identical binding sites, independent binding sites and so on and so forth.

It could so happen that the binding sites are not identical or they are dependent upon each other or there is cooperative binding, which we will see later. If we have multiple identical binding sites, then we would have a number of ligands that would actually associate with the protein. So in this case the Scatchard equation would be $v / [L] = nK_A - vK_A$.

So again when we plot $v / [L]$ versus v , we should get a straight line and from the slope of the intercept we get this and the y intercept gives us nK_A , which is useful in determining binding sites and proteins.

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For n sites on the macromolecule:

Concentration of bound ligand

$$= [PL] + 2[PL_2] + 3[PL_3] + \dots = \sum_{i=1}^n i[PL_i]$$

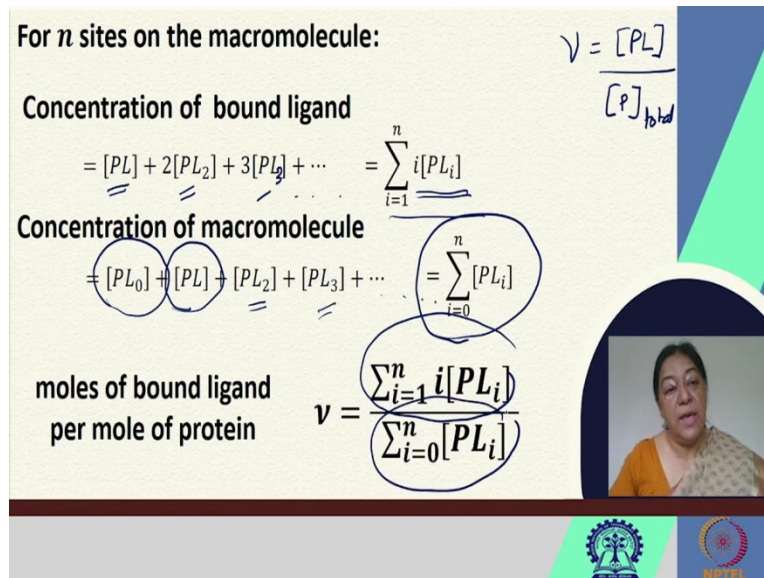
Concentration of macromolecule

$$= [PL_0] + [PL] + [PL_2] + [PL_3] + \dots = \sum_{i=0}^n [PL_i]$$

moles of bound ligand per mole of protein

$$v = \frac{\sum_{i=1}^n i[PL_i]}{\sum_{i=0}^n [PL_i]}$$

$v = \frac{[PL]}{[P]_{total}}$



So for n sites on the macro molecule, let us see how we can actually calculate the value for the v . We need the concentration of the bound ligand, that is the numerator and we need the concentration of the total protein, that is the denominator. We know that when we are looking at the concentration of bound protein, we could have 1 molecular ligand, we could have 2, we could have 3 and so on and so forth.

We have an expression like $\sum_{i=0}^n [PL_i]$ summing over all the possible interactions. When we look at the concentration of macromolecule, we have it in this form or in this form with 1 ligand bound or with 2 ligands bound or with 3 and so on and so forth.

Then the summation of the amount of macromolecule would be this. So, the moles of bound ligand per protein would be $\sum_{i=1}^n i[PL_i]$ as the numerator and $\sum_{i=0}^n [PL_i]$ as the denominator, accounting for the n sites of macromolecule, where we have the moles of bound ligand per mole of bound protein.

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Multiple Sites

$$\begin{aligned}
 P + L &\rightleftharpoons PL \Rightarrow \\
 PL + L &\rightleftharpoons PL_2 \Rightarrow \\
 PL_2 + L &\rightleftharpoons PL_3 \Rightarrow \\
 &\vdots \\
 &\vdots
 \end{aligned}$$

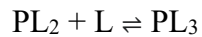
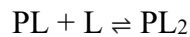
$$v = \frac{\sum_{i=1}^n i K_i [P][L]^i}{\sum_{i=0}^n K_i [P][L]^i} = \frac{\sum_{i=1}^n i K_i [L]^i}{\sum_{i=0}^n K_i [L]^i}$$

$[PL]$

ADAIR equation

For multiple sites we have:



In this expression therefore, when we know that we can rewrite the PL concentration in terms of a product of the K values with the concentrations of the protein and the ligand, this rearranges to form or to get a new value, that has the specific equilibrium constants of each equilibrium and the ligand concentrations. So, we can get an idea of how to determine the values for the equilibrium constants. This is given by the ADAIR equation.

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Benesi-Hildebrand plot


$$\frac{v}{[L]} = nK_A - vK_A$$



$$\frac{1}{v} = \frac{K_D}{n[L]} + \frac{1}{n}$$

$$v = \frac{[PL]}{[P]_{total}}$$

This is an equation of the form: $y = mx + c$ to extract n and K_D from binding data.

- m (slope): $\frac{K_D}{n}$
- c (y intercept at $x=0$): $\frac{1}{n}$



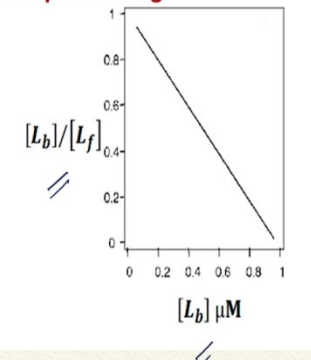



Another plot that is very commonly used is called the Benesi-Hildebrand plot, where again our definition for v is the protein ligand concentration, the complex and the total protein. Here we have $v / [L] = nK_A - v K_A$ giving us a number of binding sites n . When we rearrange this equation do a bit of algebra, we can rearrange this to give us 1 by v with 1 by L , that we can plot to give us the value of n and the value of K_D .

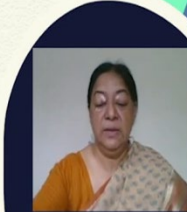
So this is an equation to the form of $y = mx + c$ and from this equation we can extract n and K_D from the binding data. We will look at specific examples in our discussion class, where we will solve for some specific problems, where we will determine the values of n and K_D .



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Simple Binding



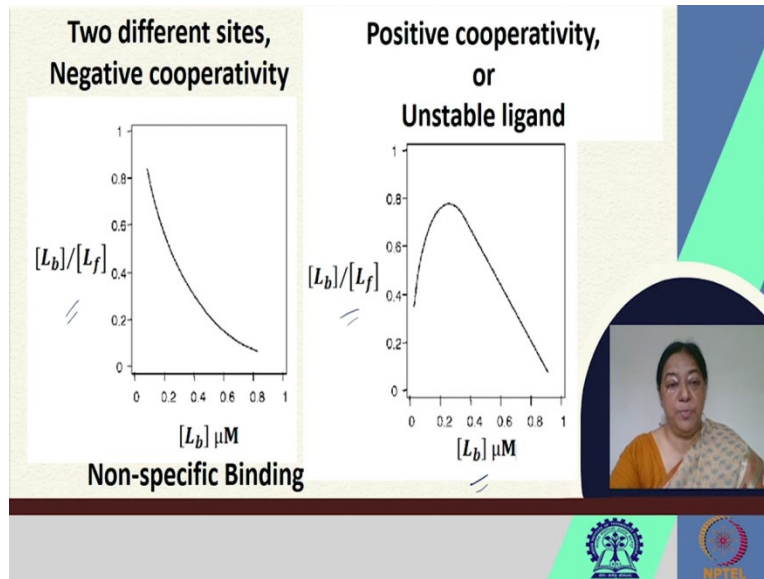
L_b and L_f are the concentrations of bound and free ligand, respectively



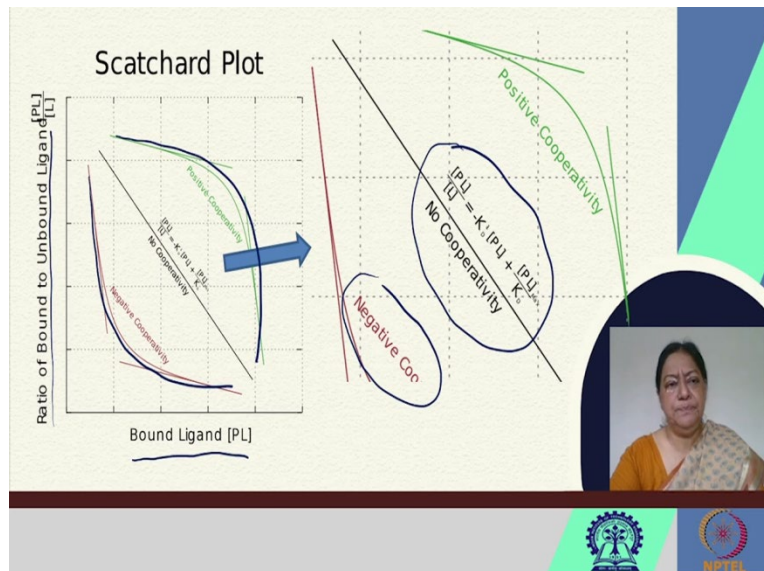
When we look at the simple binding situation, we have the free ligand versus the bound ligand. We have a simple binding given by our Scatchard equation also.

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When we look at two different sites and we see that we have a binding characteristic curve like this [refer to slide], this accounts for non specific binding given by negative cooperativity. If we look at positive cooperativity or an unstable ligand, we would get a plot like this. So simple binding plot would give us a distinct straight line, we can have variations in terms of negative cooperativity and positive cooperativity.

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A look at our Scatchard plot, where we have the ratio of the bound to the unbound ligand and the bound ligand PL concentration. When we have a straight line, that corresponds to no cooperativity, negative cooperativity if we have a line that corresponds or a curve that appears

like this or a data like this, which would indicate that there is positive cooperativity in our system. There would be different methods to deal with the positive cooperativity.

(Refer Slide Time: 28:12)

➤ Binding of ligands to a macromolecule ✓

➤ Protein ligand binding kinetics ✓

➤ Thermodynamics of protein-ligand interactions ✓

➤ Protein-ligand binding models ✓

So we looked at the bindings of ligands to the macromolecule, protein ligand binding kinetics, the thermodynamics of protein ligand interactions and specific types of models, which we will revisit later on when we look at cooperativity and see how our model changes, as to how our plots will change to understand the effect of cooperativity.

(Refer Slide Time: 28:43)

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➤ Donald Voet and Judith G. Voet Biochemistry; 4th edition

These [refer to slide] are the references.

Thank you.