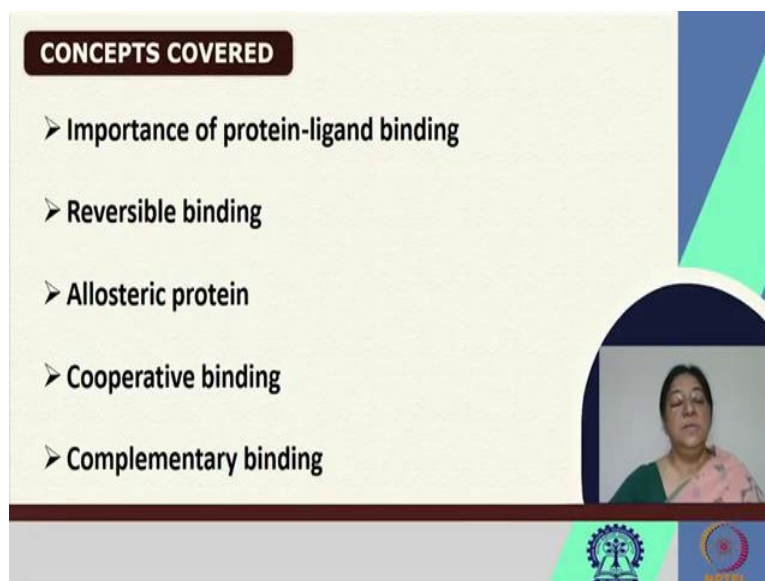


Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 05
Protein ligand interactions
Lecture - 21
Types of Protein Ligand Interactions

We begin module 5 which is going to deal with protein ligand interactions, in this course on fundamentals of protein chemistry. In the 5 lectures that we will have in this module; we will speak of the types of protein ligand interactions, the kinetics and thermodynamics, some experimental aspects, theoretical aspects and then a discussion class, where we will be looking at specific problems relating to this.

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CONCEPTS COVERED

- Importance of protein-ligand binding
- Reversible binding
- Allosteric protein
- Cooperative binding
- Complementary binding

The slide features a dark blue header with the title 'CONCEPTS COVERED' in white. Below the title, five bullet points are listed in black text. A small video inset in the bottom right corner shows Prof. Swagata Dasgupta. The slide also includes logos of IIT Kharagpur and the Department of Chemistry at the bottom.

In our discussion we will be looking at the importance of protein ligand binding, what we mean by reversible binding, allosteric proteins, cooperative and complementary binding.

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KEYWORDS

- Binding sites
- Co-factor, Allosteric
- Cooperative
- Immune response
- Binding affinity

The slide features a light green background with a dark blue header containing the word 'KEYWORDS' in white. Below the header, five bullet points with right-pointing chevrons list the keywords. A small red dot is visible in the center of the slide. In the bottom right corner, there is a circular inset showing a woman with glasses and a pink and green sari. At the bottom of the slide, there are two logos: a blue gear-like logo on the left and a red circular logo on the right.

The specific binding sites that are present in proteins is a very important part of protein ligand binding.

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Binding of ligands to a macromolecule

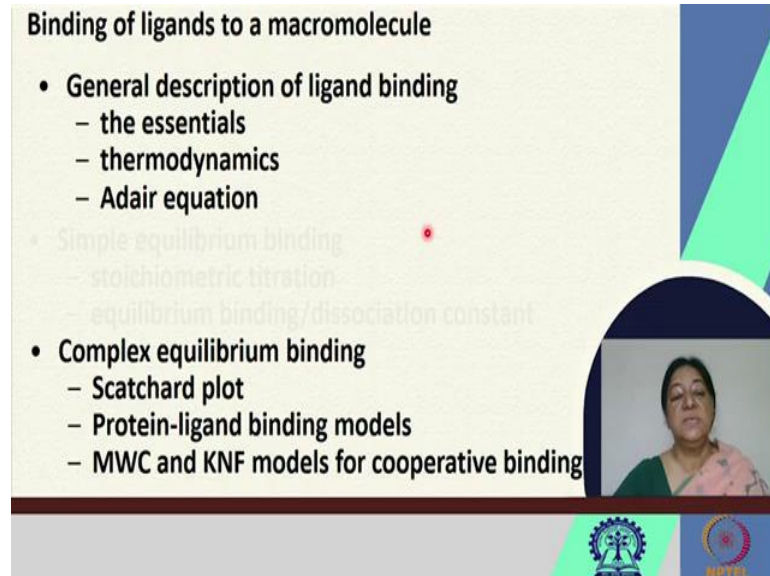
- General description of ligand binding
 - the essentials
 - thermodynamics
 - Adair equation
- Simple equilibrium binding
 - stoichiometric titration
 - equilibrium binding/dissociation constant

The slide features a light green background with a dark blue header containing the title 'Binding of ligands to a macromolecule' in white. Below the header, two main bullet points with circular markers are listed. The first bullet point has three sub-bullets with hyphens. The second bullet point has two sub-bullets with hyphens. A small red dot is visible in the center of the slide. In the bottom right corner, there is a circular inset showing a woman with glasses and a pink and green sari. At the bottom of the slide, there are two logos: a blue gear-like logo on the left and a red circular logo on the right.

In the binding of ligands to a macromolecule, our lectures will cover the general description of ligand binding, the essentials, the thermodynamics and the binding constants, the dissociation constants; that are important in determining the strength of the protein ligand binding. The equilibrium binding in terms of the stoichiometry

that occurs in protein ligand binding, the equilibrium, binding and dissociation constants.

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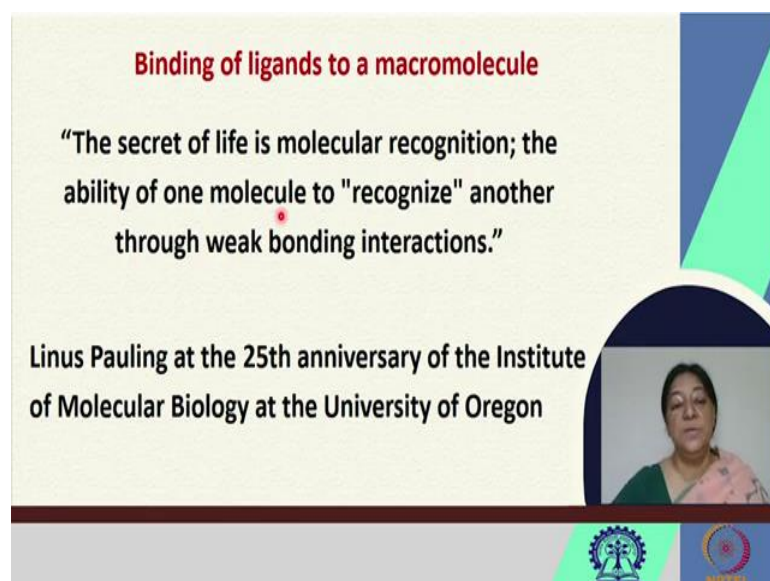
Binding of ligands to a macromolecule

- General description of ligand binding
 - the essentials
 - thermodynamics
 - Adair equation
- Simple equilibrium binding
 - stoichiometric titration
 - equilibrium binding/dissociation constant
- Complex equilibrium binding
 - Scatchard plot
 - Protein-ligand binding models
 - MWC and KNF models for cooperative binding

The slide features a video inset of a woman in the bottom right corner and logos for IIT Bombay and IIT Madras at the bottom.

This will be followed by complex equilibrium binding, where we will be looking at specific known plots such as the Scatchard plot, that give us an indication of the number of binding sites in a molecule, followed by other protein ligand binding models and models for cooperative binding.

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Binding of ligands to a macromolecule

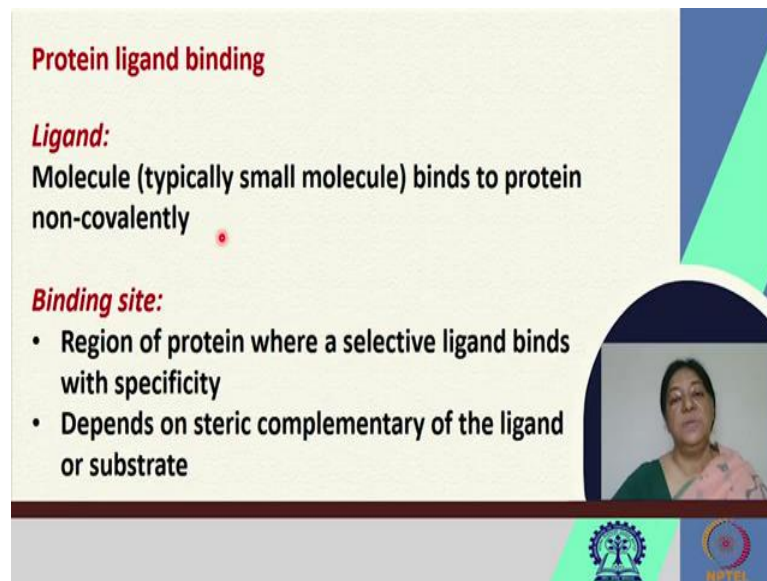
“The secret of life is molecular recognition; the ability of one molecule to "recognize" another through weak bonding interactions.”

Linus Pauling at the 25th anniversary of the Institute of Molecular Biology at the University of Oregon

The slide features a video inset of a woman in the bottom right corner and logos for IIT Bombay and IIT Madras at the bottom.

When we look at binding of ligands to a macromolecule, we remember the important quote given by Linus Pauling at the 25th anniversary of the Institute of Molecular Biology at the University of Oregon, where he mentions that “The secret of life is molecular recognition and the ability of one molecule to “recognize” another through weak bonding interactions”. This is exactly what occurs in protein ligand binding.

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Protein ligand binding

Ligand:
Molecule (typically small molecule) binds to protein non-covalently

Binding site:

- Region of protein where a selective ligand binds with specificity
- Depends on steric complementary of the ligand or substrate

The slide features a light green background with a blue and green geometric design on the right side. A small red dot is positioned next to the word 'non-covalently'. A circular inset on the right shows a woman speaking. At the bottom, there are logos for a university and a department.

When we look at protein ligand binding, we have a ligand which is typically a small molecule, that binds to the protein in a non-covalent manner. The binding site on the specific protein or macromolecule of interest, is a region of the protein where this ligand will bind a very specific binding, which is more so when we see enzyme substrate complexes later when we have our module on enzymes. This will depend upon the steric complementarity, the geometric complementarity, as well as the chemical complementarity in the active site.

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Protein ligand binding

Proteins are dynamic molecules whose functions almost invariably depend on interactions with other molecules.

Protein + ligand \rightleftharpoons Protein-ligand complex

Intermolecular interactions

- Electrostatic interactions
- van der Waals interactions
- Hydrophobic interactions

The slide features a light green background with a blue and green geometric design on the right. A small inset video shows a woman speaking. At the bottom, there are logos for IIT Bombay and NPTEL.

Proteins themselves as we know, are dynamic molecules whose functions invariably depend upon the interactions with the other molecules. Whether we have this small ligand binding to the protein or we are looking at another protein that is coming to interact in a protein-protein interaction or even protein DNA or protein RNA interaction. The interactions are important in terms of the types of interactions that can be formed. We know these are weak non-covalent types of interactions.



So we form the protein plus the ligand; we form the protein ligand complex. In the formation of the complex, we have these intermolecular interactions, that can be electrostatic interactions, Van der Waals interactions or hydrophobic interactions. As we have seen before, when we have looked at the protein folding characteristics that occurred in the specific interactions or the folding interactions between the amino acids and the overall folding.

In this case we are looking at a small molecule, a ligand that is coming to bind with the protein at a specific site on the protein.

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Protein-ligand interactions are crucial to biological function

- Communication (hormones, neurotransmitters, second messengers, downstream regulators)
- Protein trafficking (e.g. PKC attachment to membrane by binding to DAG)
- Prosthetic groups (e.g. heme, retinal)
- Defense/offense (e.g. bacterial toxins)



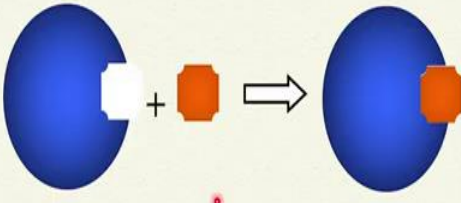


The protein ligand interactions are crucial to biological function. They function in communication such as hormones, neurotransmitters, second messengers, downstream regulators, in protein trafficking, prosthetic groups and defense/offense, where we have bacterial toxins come into the picture.

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Protein-ligand binding models

1. Lock & key

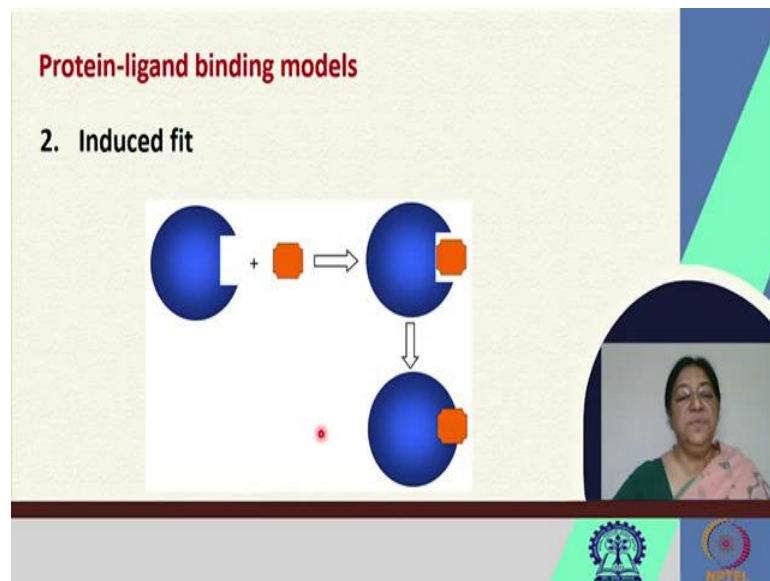


When we look at the different binding models that are possible for proteins, a common model is the lock and key model, which is common more so in enzyme substrate. But similarly it is there in protein ligand binding models. The enzyme

substrate interactions or the enzyme substrate binding is a subset of a protein ligand binding.

What we have in this case is we have a typical active site on the protein and we have on the ligand of the small molecule a perfect match to the active site, giving us a lock and key method given by Emil Fischer long time ago; in the lock and key method of protein ligand binding or enzyme substrate binding.

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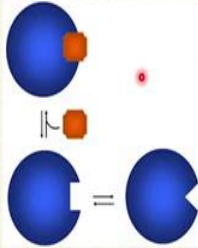


In terms of an induced fit model, when we have a specific active site, it remodels itself to accommodate the substrate. This is Koshland model where we look at the active site residues that remodel themselves or orient themselves in such a fashion, so that the substrate can be accommodated into the active site.

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Protein-ligand binding models

3. Conformational selection
Proteins shift spontaneously
between different conformations

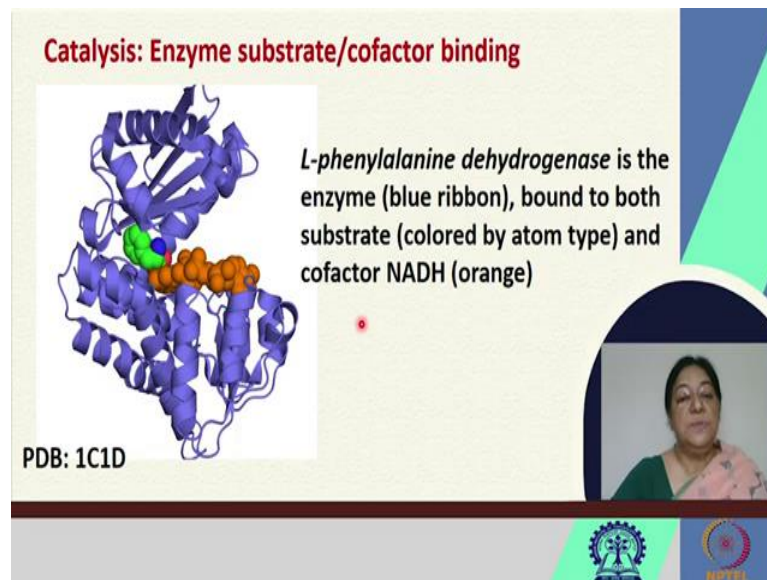


The diagram illustrates the conformational selection model. It shows a blue protein sphere in two different conformations, separated by a double-headed arrow indicating equilibrium. In the top conformation, the protein has a specific pocket shape. An orange ligand is shown binding to this pocket. In the bottom conformation, the protein has a different pocket shape. A red dot represents the ligand. A small inset video shows a woman speaking.

Another case that we look at, is a conformational selection where the proteins can shift spontaneously between different conformations. In this case what we can have is, we can have a specific fit or it may so happen that we have another confirmation and as the substrate come, there is an equilibrium between this [refer to slide] confirmation and the other confirmation, where the active site residues have a different geometric pattern to them.

This is followed by the appearance of the ligand, following which there is an accommodation of the ligand into the specific pocket, where the ligand binding will occur.

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Let us look at some specific examples. Here [refer to slide] we are looking at a catalysis. An enzyme substrate and cofactor binding, an extremely important aspect of protein ligand binding in general. In this case we are looking at L-phenylalanine dehydrogenase; once we get to our enzyme discussions we will know what this dehydrogenase means. This is the enzyme that is given in the blue ribbon here.

We recognize from the structure, that these are the strands that form the β -sheet and these are the helices that are there in the protein. And these connectors are the random coils or the specific turns or linkers that link the secondary structure elements together. Here, we see that there is a cofactor which is NADH that is coloured in orange.

In this case the substrate that is coloured by the atom type, which is L-phenylalanine is present at the active site and we have both the substrate and the cofactor bound to this specific enzyme. This binding will occur through non covalent interactions. Particularly for enzyme substrate complexes because for the reaction to occur in the product formation as we will see later on, we do not want the enzyme to bind to the substrate too strongly because, then it will not release the substrate or will not release the product formed, if the binding is too tight. However, for the design of inhibitors, we would like a strong binding to occur.

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Regulation:
Enzyme-activator/inhibitor, receptor-agonist/antagonist

Allosteric regulators:
Fructose-1,6-bisphosphatase with 2,5-anhydro-D-glucitol-1,6-bisphosphate (AHG, a substrate analogue) and AMP (an allosteric inhibitor)

Allosteric ligand occupies a distinct binding site – distant from the catalytic site.

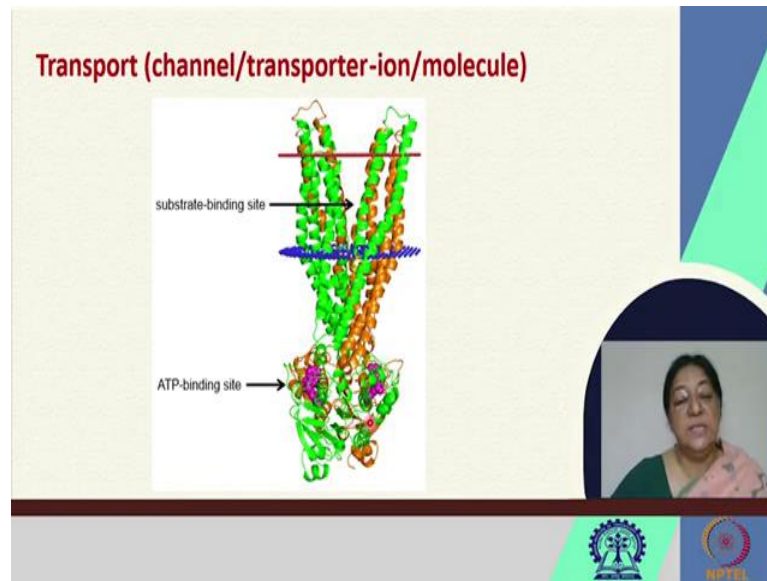
An enzyme activator inhibitor in this case, will also be looking at allosteric regulators, where what we mean by this is a binding to a site that is not the active site of the protein. Now this has important implications in a regulation of the active site.

The active site residues may not bind to the activator or inhibitor. It would be another site on the protein or the enzyme, that would bind to this regulator that would in turn affect the functionality of the enzyme in terms of its binding to the substrate.

In this [refer to slide] particular picture figure we see fructose-1,6-bisphosphatase with 2,5-anhydro-D-glucitol-1,6-bisphosphate marked with AHG here, which is a substrate analogue and AMP, which is an allosteric inhibitor. If this is a substrate analogue this means that this is binding to the active site.

But it is not the substrate, it is an analogue of the substrate. And this AMP is an allosteric inhibitor binding to a site other than the active site, but it is going to affect the binding of the substrate.

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So, the allosteric ligand occupies a distinct binding site that is distant from the catalytic site. We look at transporters; channel transporters or ions or molecules. Here [refer to slide] we have a ATP binding site. A specific substrate binding site on this molecule, where we again see that the ATP binding site is remote from the substrate binding site.

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Key aspects in protein-ligand binding: AFFINITY & SPECIFICITY

- **Affinity**
 - Types of complexes
 - Measuring and calculating the binding energy
 - Thermodynamics
- **Specificity**
 - Models
 - Determinants
 - Promiscuity

The key aspects in protein ligand binding are the affinity - how strong is the binding and its specificity - how specific is the binding. In the affinity there are different

types of complexes that may be formed and it is important to understand how the binding energy may be measured and calculated.

So, we need to understand the thermodynamics of the specific binding. For the specificity there are specific models, determinants and promiscuity where we can design analogues. That if we know a particular function of the protein, knowing say for an enzyme we know the specific substrate, we can prepare substrate analogues that would then bind to the active site, acting as inhibitors.

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The transient nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic circumstances.

Reversible binding of a protein to a ligand

Allosteric binding

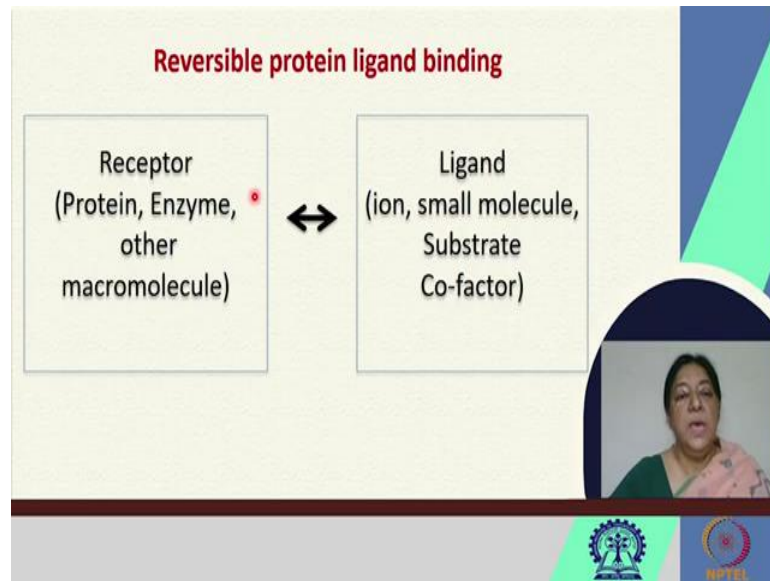
Cooperative binding of a protein to a ligand

Complementary binding of a protein to a ligand

Now, this transient nature of protein ligand interactions is critical to life, because it allows an organism to respond rapidly and reversibly, to changing environment and metabolic circumstances. So here we look at reversible binding of a protein to a ligand, because we would want our protein to function, to be available for another ligand to bind to it.

We look at allosteric binding, where we have the small molecule bind to a site other than the active site of the protein. A cooperative binding of a protein to a ligand and a complementary binding of a protein to a ligand. In all these types of binding we look at a transient nature that occurs due to the non-covalent nature of the binding that is observed.

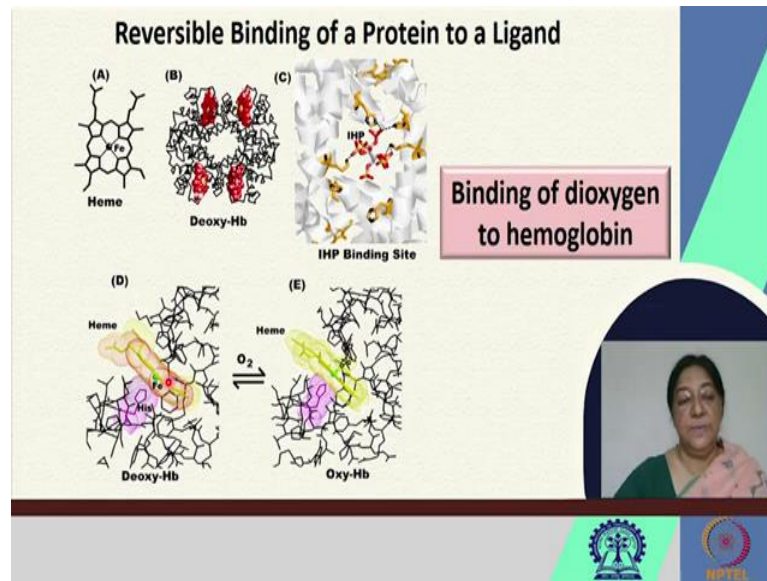
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In reversible protein ligand binding, our receptor that could be the protein could be an enzyme or any other macromolecule say for example, DNA. We have a small ligand relatively small molecule, that could be an ion, in ion transport, could be a small molecule, could be a substrate where we have enzymes as our receptors or could be a cofactor.

This reversibility is important so that we have our enzyme or our protein or our other macromolecule available to other ions or small molecules or substrates or cofactors; so that we have a continuous biochemical process that occurs. The biochemical reactions may continue to occur because of the reversible protein ligand binding, making our receptor available for other small molecules bind.

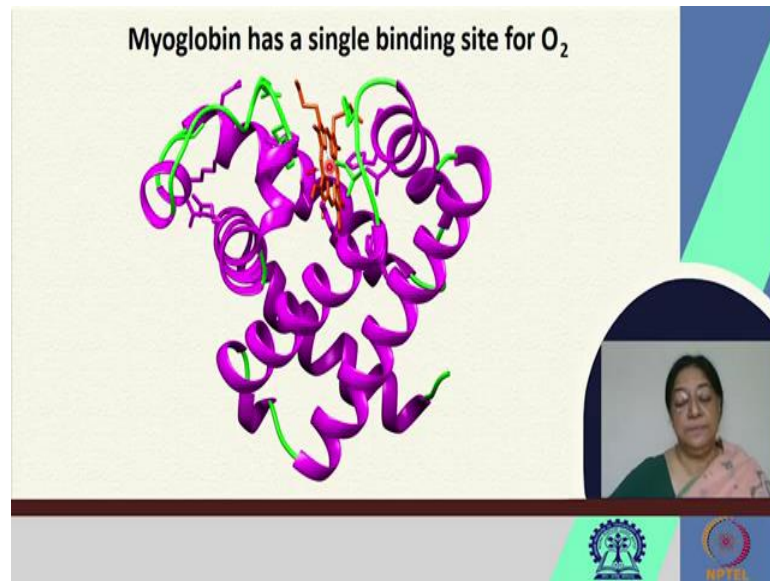
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The most common example of reversible binding of a protein to a ligand, is the binding of oxygen to hemoglobin. We have deoxyhemoglobin, we know that hemoglobin is a tetrameric molecule with 2α and 2β subunits. Each of them have a heme group which has an iron. So, [refer to slide] these are the Heme groups that are marked in red here and here we have deoxyhemoglobin, that does not have oxygen bound to it.

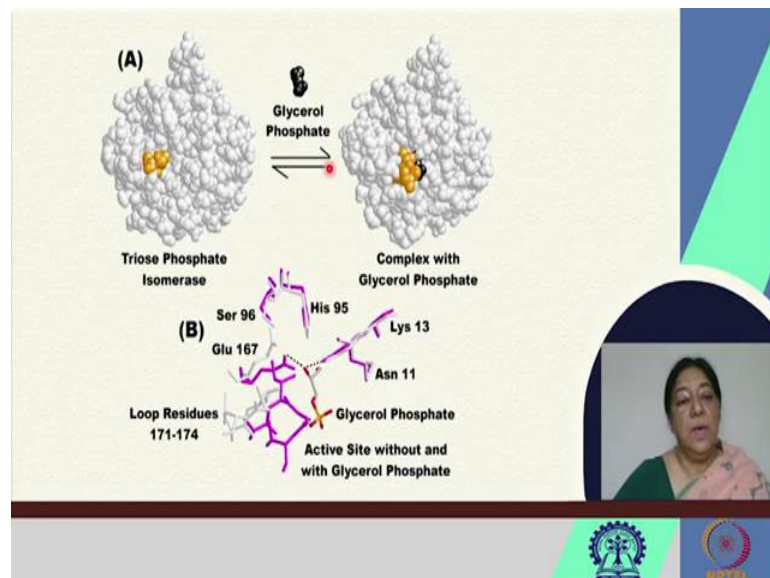
Now we have a specific IHP binding site. But if we look at the binding of dioxygen to hemoglobin we will see, that this occupies a site present here and the oxygen binds to a site that is near the iron atom of the heme moiety.

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Myoglobin, which is a monomeric protein on the other hand has a single binding site for the oxygen. Here we see the heme for the myoglobin and this is the iron atom.

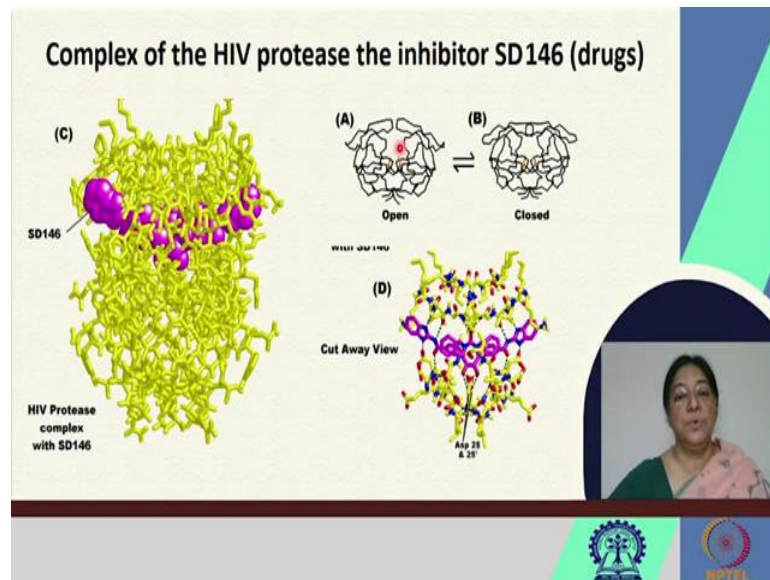
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Another example is triose phosphate isomerase, where we have a complex with the glycerol phosphate. So, there is the active site and we have the cleft where we have the molecules bound. In specific cases like this, we have active site residues that are involved in the recognition.

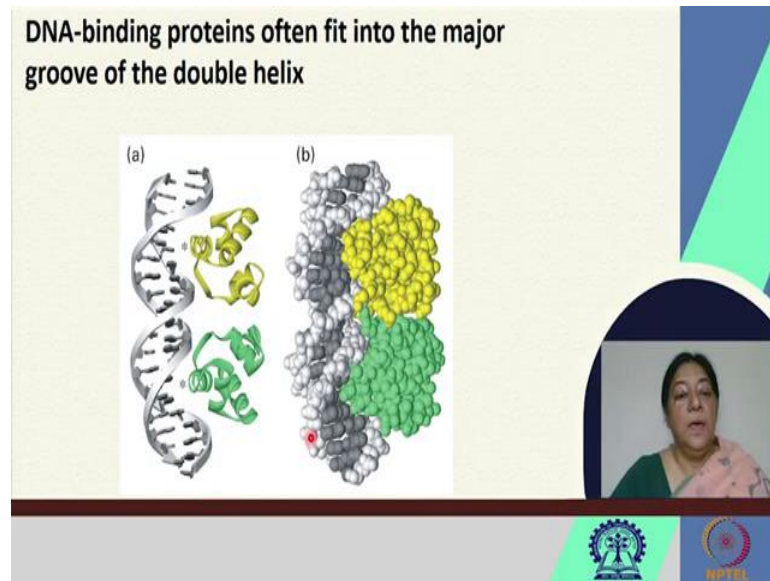
As we will look at specific enzyme mechanisms, we will see that there are specific sites that recognize specific moieties on the substrate molecule, be it a sugar molecule or a base or a phosphate. In this case it could be the phosphate, which is recognized by the active site in the binding of this compound.

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When we look at a complex of a protein with even drugs, for example in this particular diagram here, we are looking at the HIV protease complex with SD 146, which is a drug. The idea is looking at the open and closed format and seeing how the binding of the drug is going to affect this conformational change that is essential for the activity. This is protein ligand binding.

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We can also look at DNA binding, where we have DNA binding proteins that often fit into the major groove of the double helix as we can see here [refer to slide], where we know that we have a sugar phosphate backbone and these are the bases. A study of the nucleic acid structure tells us that this is highly negatively charged because of the phosphate groups. And we have these bound proteins to the major grooves of the double helix, giving us again a protein ligand binding format, where our ligand in this case is the DNA.

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Allosteric Protein:

- An allosteric protein is one in which the binding of a ligand to one site affects the binding properties of another site on the same protein.
- The term “allosteric” derives from the Greek *allos*, “other,” and *stereos*, “solid” or “shape.”



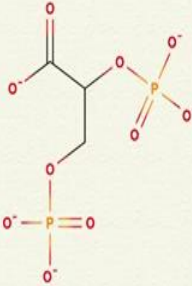
An allosteric protein is one, where we have the binding of a ligand to one site that affects the binding properties on another site on the same protein. In this case the active site which is available for the substrate or for the specific ligand, that is essential in the binding, for the function of the protein to be performed.

We have now another small molecule that is bound to another site, that affects the binding of the ligand to the protein. So, the term “allosteric” derives from the Greek allos, that is “other”, and stereos that is “solid” or “shape”. It is another area in which we have a binding of another small molecule, that is going to affect the binding of the original substrate or ligand, or the common substrate or ligand to the protein.



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**Oxygen binding to Hemoglobin:
Regulation by 2,3-Bisphosphoglycerate**

2,3-Bisphosphoglycerate is known to greatly reduce the affinity of hemoglobin for oxygen — there is an inverse relationship between the binding of O₂ and the binding of BPG.



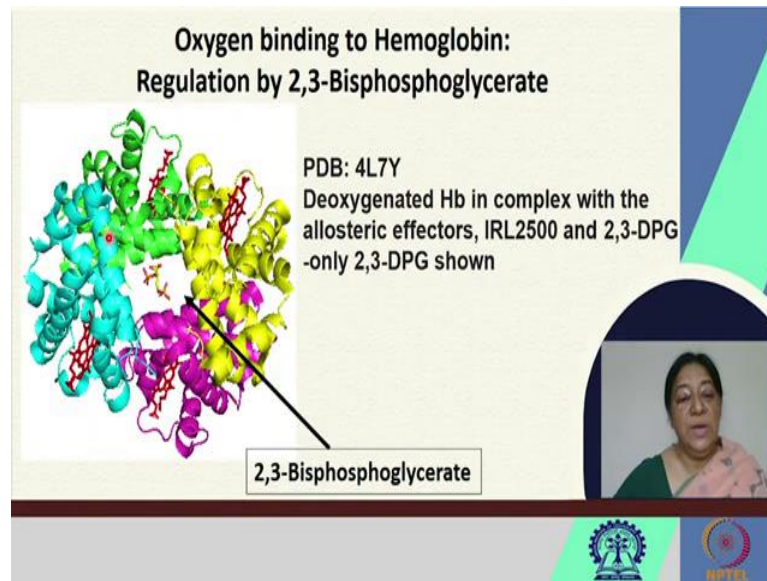
2,3-Bisphosphoglycerate



One such example is 2,3-bisphosphoglycerate. This is a molecule that greatly reduces the affinity of hemoglobin for oxygen. There is an inverse relationship between the binding of oxygen and the binding of BPG as this is called. This [refer to slide] is the structure of 2,3-bisphosphoglycerate and it is interesting to note that the presence of this molecule reduces the affinity of hemoglobin for oxygen.

So our body responds in a manner, when there is less oxygen available. Say for example at increased heights, where the level of oxygen is low, bisphosphoglycerate is formed to reduce the affinity of hemoglobin for oxygen.

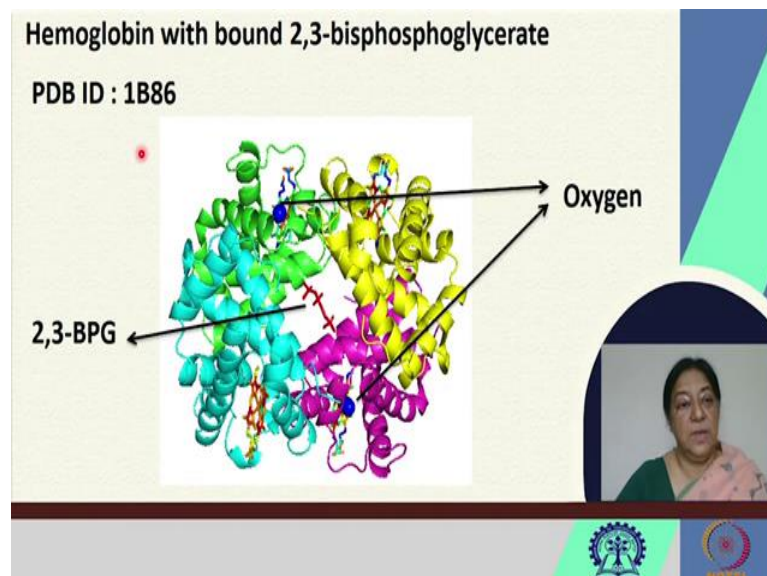
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If we look at the structure of where the bisphosphoglycerate binds, it is different from where oxygen binds. So there is a regulation by the BPG in this particular structure, where we are looking at the heme moieties that would bind the oxygen. The presence of the 2,3 bisphosphoglycerate here or diphosphoglycerate as it is also called, reduces the affinity for oxygen by hemoglobin.

We will look at the binding of this. It is an extremely interesting way that nature creates this reduction in the affinity for oxygen, when oxygen levels are low.

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Here is where we have oxygen bound and here [refer to slide] we can see where 2,3-BPG is bound. Which means that this is binding to a site, that is not affecting the binding of oxygen. But nevertheless, reduces the affinity of hemoglobin to bind the oxygen.

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Complementary interactions between proteins and ligands: The Immune system and Immunoglobulins

- ✓ immune system eliminates viruses, bacteria, and other pathogens and molecules that may pose a threat to the organism.
- ✓ On a physiological level, the immune response is an intricate and coordinated set of interactions among many classes of proteins, molecules, and cell types.

The slide features a video inset of a woman in the bottom right corner and logos for IIT Bombay and NPTEL at the bottom.

When we look at complementary interactions between proteins and ligands, a very important aspect is the immune system and immunoglobulins, antibody antigen interactions, which we will study in greater detail when we look at protein-protein interactions, large molecule interactions. The immune system is extremely important in the elimination of viruses, bacteria and other pathogens and molecules that may pose a threat to the organism. This is where our immune system comes to play.

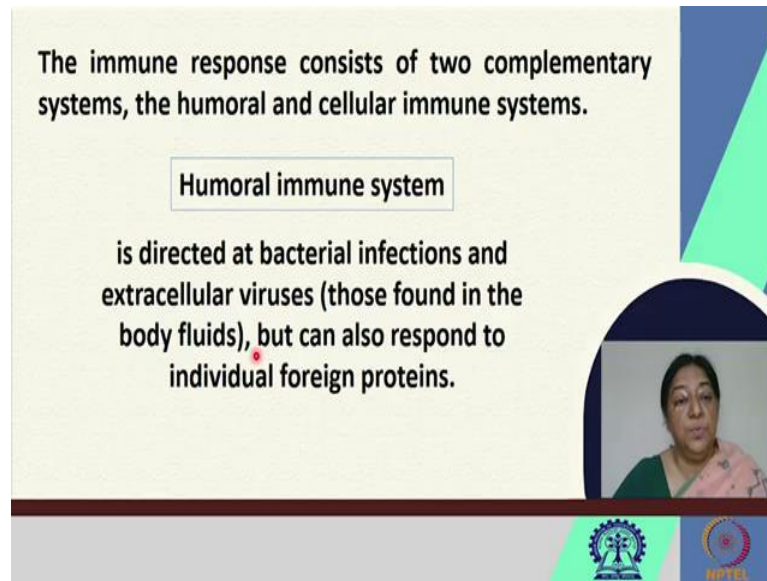
On a physiological level, the immune system is actually an intricate or the immune response that is triggered, is an intricate and coordinated set of interactions among many classes of proteins, molecules, and cell types. Now an understanding of the modes of action, is beyond this course in the terms of how the T cells and the B cells are triggered in an immune response. But the fact that we have a protein ligand binding, a specific interaction that occurs at the molecular level is of interest to us.

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The immune response consists of two complementary systems, the humoral and cellular immune systems.

Humoral immune system

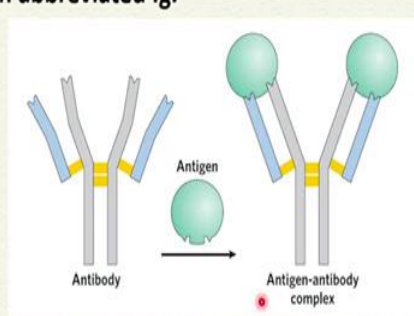
is directed at bacterial infections and extracellular viruses (those found in the body fluids), but can also respond to individual foreign proteins.



The immune response consists of two complementary systems; one is the humoral system and the other is a cellular immune system. In the humoral immune system, this is directed at bacterial infections and extracellular viruses that are found in body fluids, but could also respond to individual foreign proteins.

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At the heart of the humoral immune response are soluble proteins called antibodies or immunoglobulins, often abbreviated Ig.

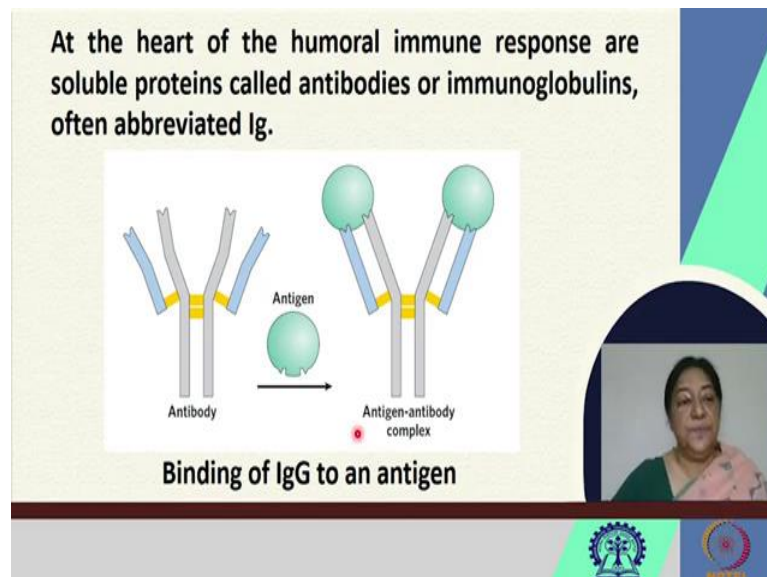


Antibody

Antigen

Antigen-antibody complex

Binding of IgG to an antigen

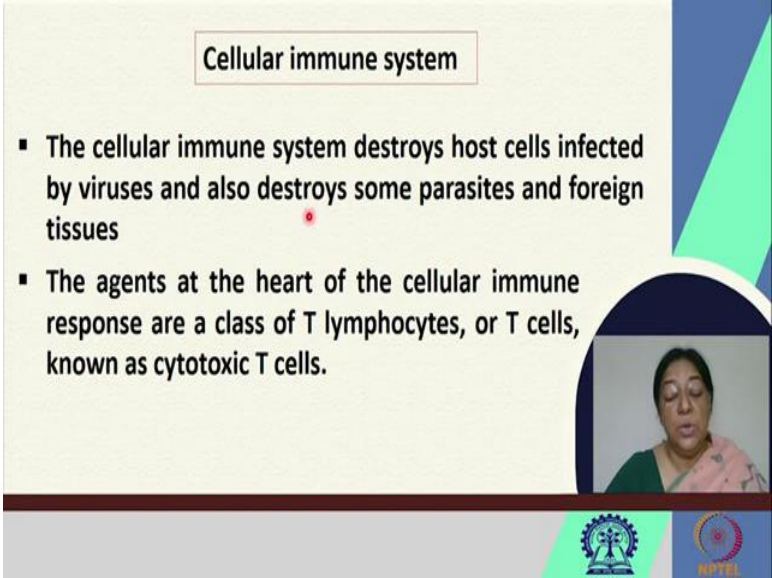


At the heart of the humoral immune response are the soluble proteins called antibodies or immunoglobulins, often abbreviated as Ig. There are different types of immunoglobulins and what we have here [refer to slide] is a diagram of this specific

immunoglobulin, where we are looking at IgG immunoglobulin G, that is binding to an antigen, which is the small molecule that we see here.

Now, what is important is the structure of the antibody is such, it is a y shaped molecule, with specific disulphide linkages and the geometric complementarity that we see here in the binding of the antigen to the antibody, is extremely important in forming the tight antigen antibody complex that is extremely important to fight of disease.

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Cellular immune system

- The cellular immune system destroys host cells infected by viruses and also destroys some parasites and foreign tissues
- The agents at the heart of the cellular immune response are a class of T lymphocytes, or T cells, known as cytotoxic T cells.

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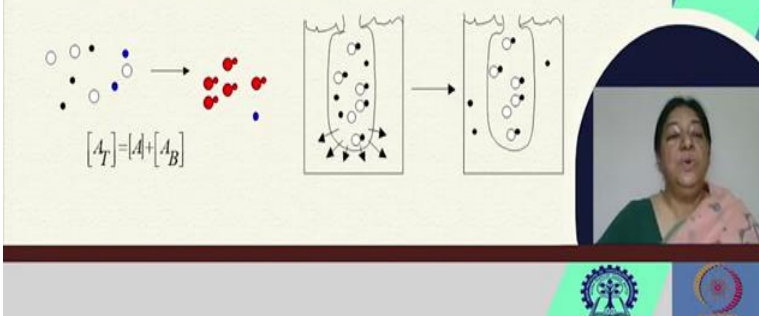
When you look at a cellular immune system, we are looking at a system that destroys host cells infected by viruses and also destroys parasites and foreign tissues. The agents at the heart of the cellular immune response are a class of T cells that are known as cytotoxic T cells. Cytotoxics means they are toxic to the cells.

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Experimental Measurements of Binding

Equilibrium dialysis:
 A_T = total amount of substrate
 A_B = total amount of BOUND substrate
 A = total amount of UNBOUND substrate

$[A_T] = [A] + [A_B]$



The diagram illustrates the equilibrium dialysis method. On the left, a mixture of substrate molecules (represented by small circles) is shown. An arrow points to a dialysis bag containing a macromolecule (large circle) and substrate. The bag is placed in a solution. Substrate molecules can move in and out of the bag through a semi-permeable membrane, while the macromolecule cannot. The diagram shows the equilibrium between bound and unbound substrate. A small inset video shows a woman speaking.

Now how do we measure all this binding? The binding can be measured by a method known as equilibrium dialysis. In this case as an experimentalist, we know the total amount of substrate; we know the total amount of bound substrate. Now, there can be specific biophysical techniques. For example, we could have a specific absorbance, a specific signal from the molecule that would tell us, that there is a conformational change due to the binding of a ligand with the substrate.

If for example, we had a tryptophan molecule or a tryptophan amino acid present at the active site of a protein or at a cleft in the protein and we are interested to know whether our ligand is binding to that specific site. We would look at a fluorescence signal. The fluorescent signal for the protein in the unbound state, would give us a complete signal that would be quenched when the ligand would bind to the specific site where the tryptophan moiety is present.

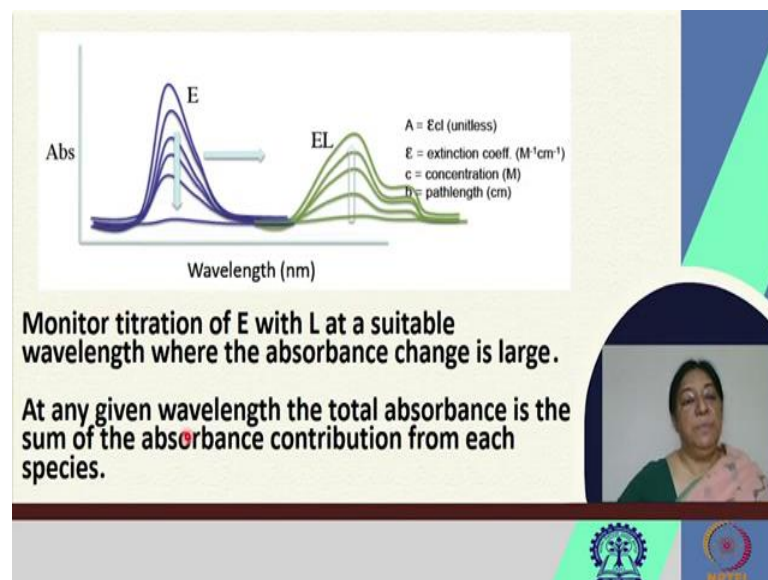
We will see these when we look at the specific binding in terms of the equilibrium binding kinetics and thermodynamics, as to how the ligand binds to our macromolecule of interest. In an equilibrium dialysis method, we had looked at the method of dialysis in a previous lecture, where we looked at protein purification. The specific dialysis membrane is such that it has a molecular weight cut off.

Our macromolecule of interest is present in the dialysis bag. Our bound, our unbound substrate is in the solution that surrounds it. Placing our bag with the

macromolecule in this particular solution allows the substrate molecules, because they are smaller in size, to penetrate this dialysis membrane and bind to our macromolecule.

Based on observations we can determine many aspects. One is we can see whether the enzyme or our protein has a bound substrate to it, by a spectroscopic signal. We can look at the concentration of the substrate outside the bag, which would tell us the equilibrium amount of unbound substrate. Given that we know the total amount of substrate, we can find the total amount of bound substrate; because we know that the total amount of substrate is either free or in the bound state.

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So at a specific example, where we have say an enzyme and we have a ligand that is going to bind to it, we are looking at a signal of absorbance at a specific wavelength. As the ligand is gradually bound to the enzyme, there is another signal that emerges. What can be done in this case is what is called a titration.

This would be the pure enzyme or the pure macromolecule. As we add the ligand, we have a reduction in the intensity at this particular wavelength. And we have an intensity of the enzyme ligand complex emerge and increase as the ligand is bound to the enzyme. This can be monitored.

We can monitor the titration of the enzyme with the ligand at a suitable wavelength, where the absorbance change is large. And at any given wavelength, the total absorbance is the sum of the absorbance contribution from each of the species that we have. In this way we can monitor and we can see how we can actually determine the binding constants, which will be the subject of the next lecture.

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Binding of ligands to a macromolecule

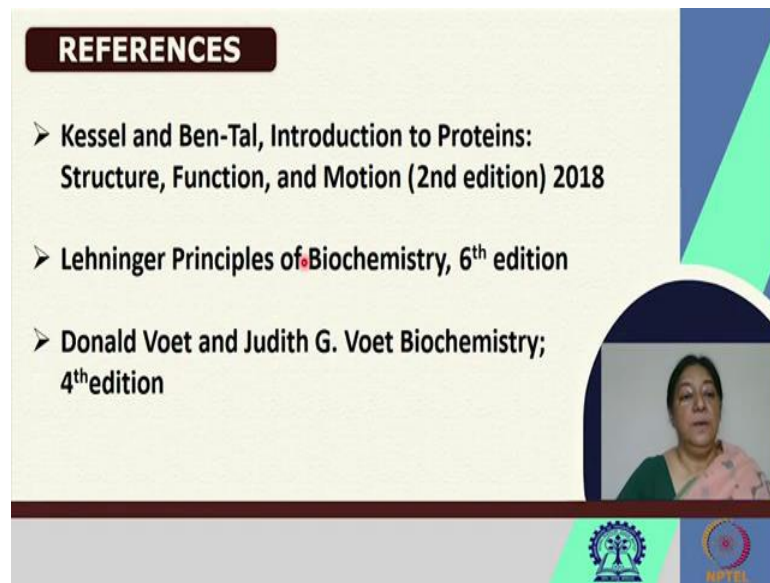
- **General description of ligand binding**
 - the essentials
 - thermodynamics
 - Adair equation
- **Simple equilibrium binding**
 - stoichiometric titration
 - equilibrium binding/dissociation constant
- **Complex equilibrium binding**
 - Scatchard plot
 - Protein-ligand binding models
 - MWC and KNF models for cooperative binding

The slide features a video inset of a woman speaking in the bottom right corner. At the bottom of the slide, there are two logos: the Indian Institute of Technology (IIT) logo on the left and the IITM logo on the right.

So if we look at the bindings of the ligands to the macromolecule, we have a general description. The essentials in the knowledge that we know, we have non-covalent interactions that contribute to this ligand binding. And we will be looking at what we mean by equilibrium binding, the stoichiometric titrations and how we can determine the equilibrium binding or dissociation constants.

And we can also look at some complex binding situations, where we could have a larger number of binding sites, which would give us different protein ligand binding models and also cooperative binding.

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REFERENCES

- Kessel and Ben-Tal, Introduction to Proteins: Structure, Function, and Motion (2nd edition) 2018
- Lehninger Principles of Biochemistry, 6th edition
- Donald Voet and Judith G. Voet Biochemistry; 4th edition

The slide features a light beige background with a dark blue header bar containing the word 'REFERENCES' in white. A list of three references is presented with blue arrowheads. On the right side, there is a vertical decorative element with blue and green geometric shapes. A circular video inset in the lower right shows a woman with dark hair wearing a green top and a pink shawl. At the bottom of the slide, there are two logos: a blue gear-like emblem on the left and a red circular emblem on the right.

These [refer to slide] are the references.

Thank you.