Fundamentals of Protein Chemistry

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Module - 06
Enzymes and Enzyme Mechanisms
Lecture - 26
Enzymes I: Classification

We begin our discussion on module 6, that is going to deal with enzymes and enzyme mechanisms. In the first part of this module, we will be talking about the classification of enzymes.

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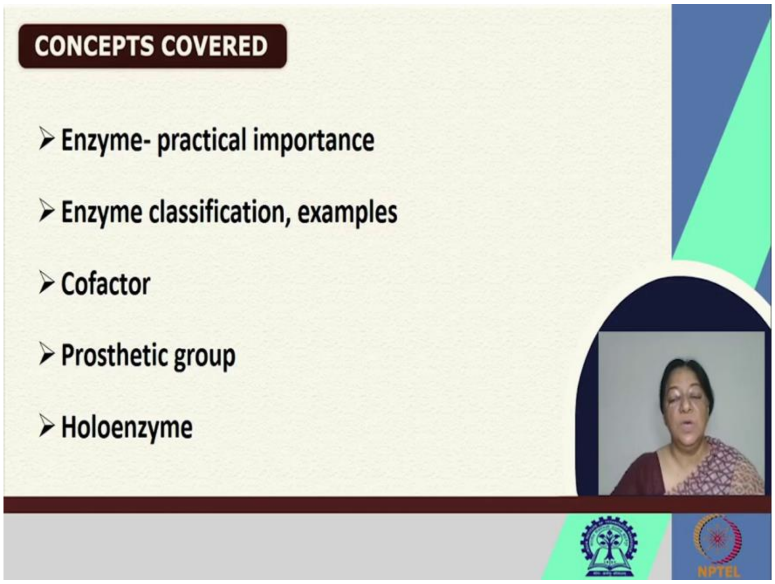

CONCEPTS COVERED

- Enzyme- practical importance
- Enzyme classification, examples
- Cofactor
- Prosthetic group
- Holoenzyme

Before we go into the classification of enzymes, we need to know the practical importance of enzymes. In our discussion on protein ligand interactions, we refer to the enzyme substrate complex many times, in a subset of what we would call protein ligand interactions.

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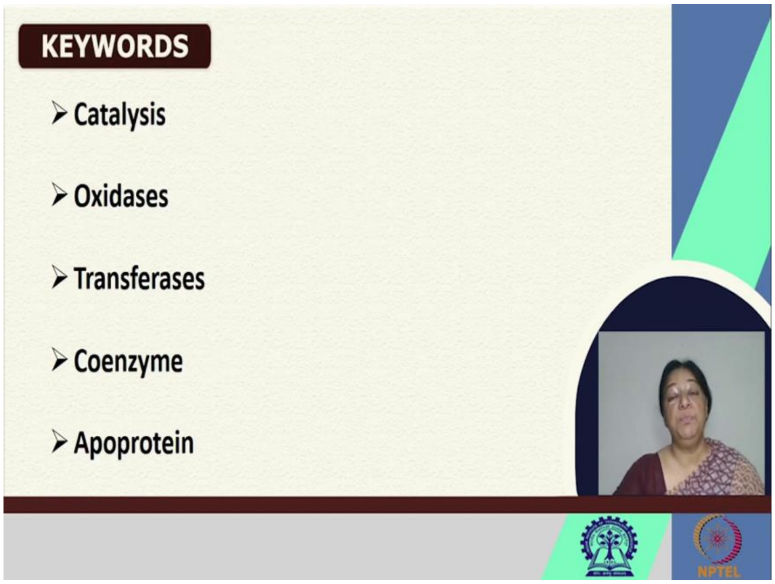

KEYWORDS

- Catalysis
- Oxidases
- Transferases
- Coenzyme
- Apoprotein

But they are very important in the processes that go on in biochemical systems, in all living organisms. The major role of enzymes is catalysis and we will see a set of reactions based on the specific enzymes.

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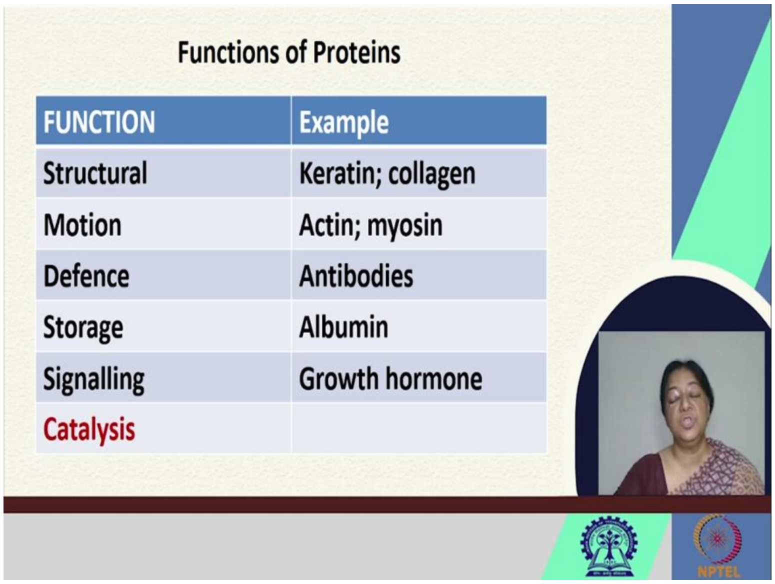

Functions of Proteins

FUNCTION	Example
Structural	Keratin; collagen
Motion	Actin; myosin
Defence	Antibodies
Storage	Albumin
Signalling	Growth hormone
Catalysis	

If we look at the functions of proteins in general, they have structural functions, functions related to motion, to defense, to storage and signalling, but we also have functions related to catalysis that are brought about by enzymes.

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Enzymes, the most remarkable and highly specialized proteins, are central to every biochemical process.

- have extraordinary catalytic power - they catalyze hundreds of stepwise reactions that degrade nutrient molecules, conserve and transform chemical energy
- make biological macromolecules from simple precursors
- have a high degree of specificity for their substrates

These enzymes are remarkable and very specialized proteins, that are central to every biochemical process. The catalytic power that these enzymes possess is very high and they have the ability to catalyze several hundreds and thousands of reactions, where they degrade the nutrient molecules, they conserve, they transform chemical energy and their specificity and rate of activity is what we are going to see in the specific enzyme mechanisms and in the overall aspect of enzyme kinetics, as to how it works to achieve the specificity and it's very high efficiency.

It can make biological macromolecules from simple precursors and have a high degree of specificity for their substrates.

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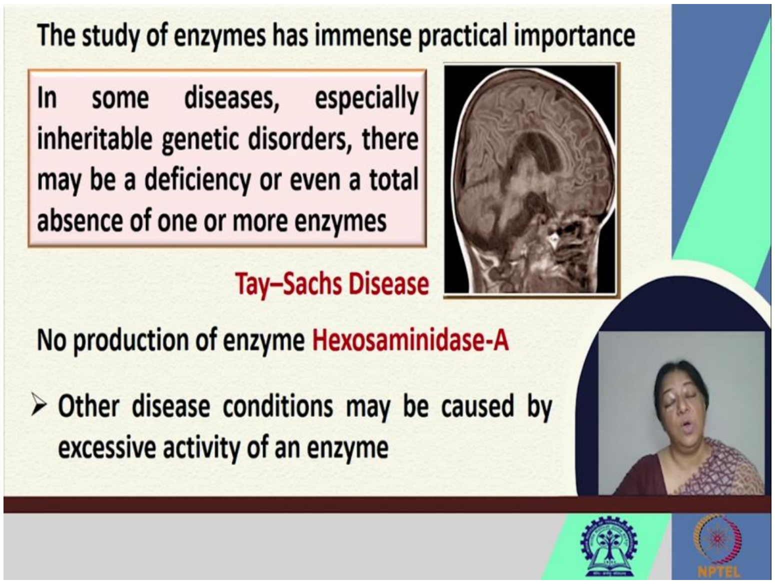

The study of enzymes has immense practical importance

In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes

Tay–Sachs Disease

No production of enzyme **Hexosaminidase-A**

- ➢ Other disease conditions may be caused by excessive activity of an enzyme

The immense practical importance of enzymes, can be understood in certain diseases particularly inheritable genetic diseases. It may so happen that there is a deficiency or even a total absence of one or more enzymes. For example, in Tay-Sachs disease there is no production of a specific enzyme Hexosaminidase-A.

Other disease conditions are caused by excessive activity of an enzyme or the absence of an enzyme or a reduction in activity of the enzyme.

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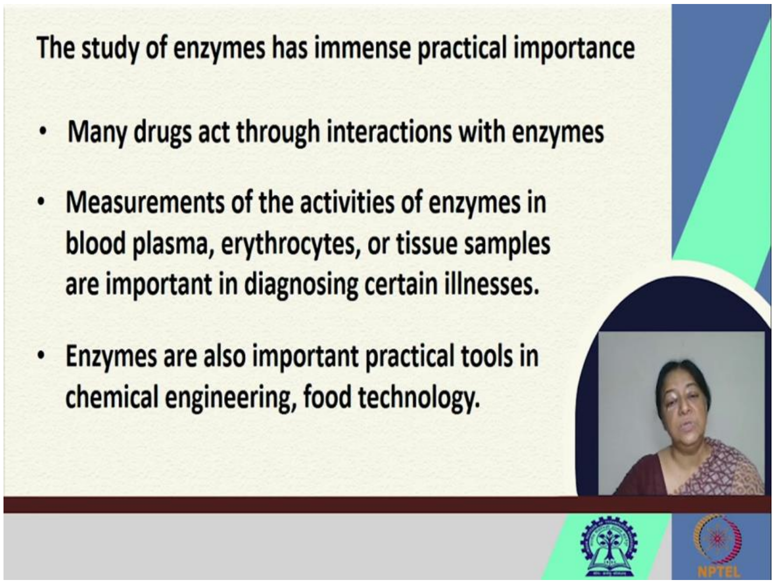

The study of enzymes has immense practical importance

- Many drugs act through interactions with enzymes
- Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses.
- Enzymes are also important practical tools in chemical engineering, food technology.

Drugs that treat diseases, also act through interactions with enzymes. The measurements of the activities of enzymes in blood plasma or any tissue, are important in the diagnosis of certain illness and today they are very important practical tools in chemical engineering and food technology.

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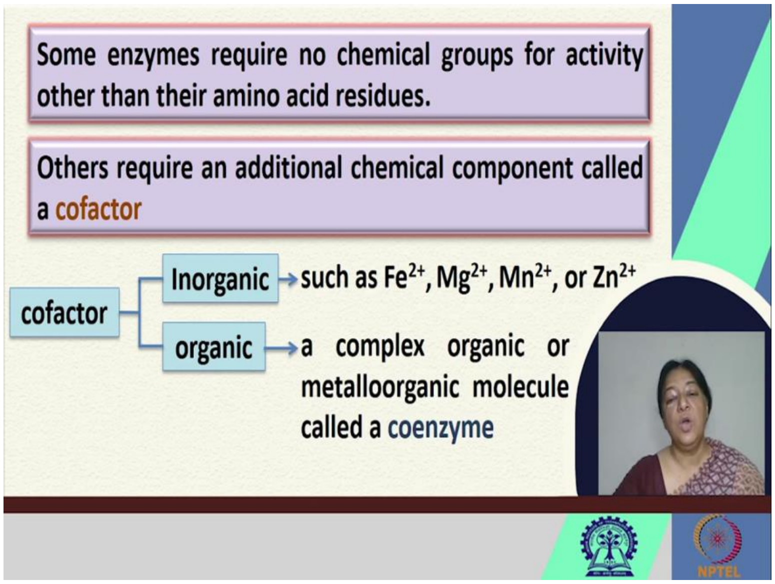

Some enzymes require no chemical groups for activity other than their amino acid residues.

Others require an additional chemical component called a cofactor

cofactor

Inorganic → such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}

organic → a complex organic or metalloorganic molecule called a coenzyme

Some enzymes do not require any additional chemical groups for their activity, other than the sequence of amino acid residues present.

Some other enzymes require an additional chemical component called a cofactor. This cofactor can be either inorganic in nature or organic in nature. For the inorganic type, we have the metal ions and for the organic type, we have complex organic or even metallo organic molecules that are called coenzymes.

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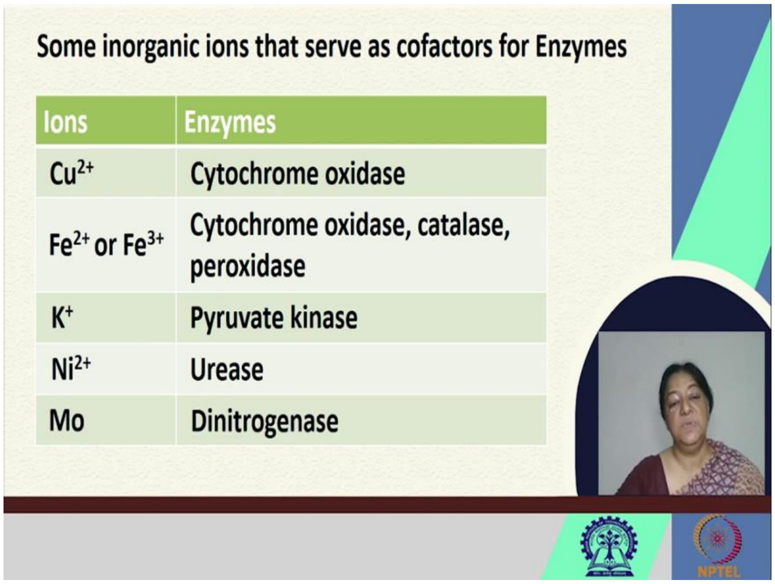

Some inorganic ions that serve as cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Ni^{2+}	Urease
Mo	Dinitrogenase

Some inorganic ions that can serve as cofactors for enzymes are copper in cytochrome oxidase, the iron in cytochrome oxidase, catalase, peroxidase and of course in hemoglobin as well, where that would be a transport protein in this type of enzyme. And another thing we need to notice is each of these enzymes, has a name that is an –ase; a catalase, a peroxidase, a kinase. In this case we have nickel in urease, molybdenum dinitrogenase.

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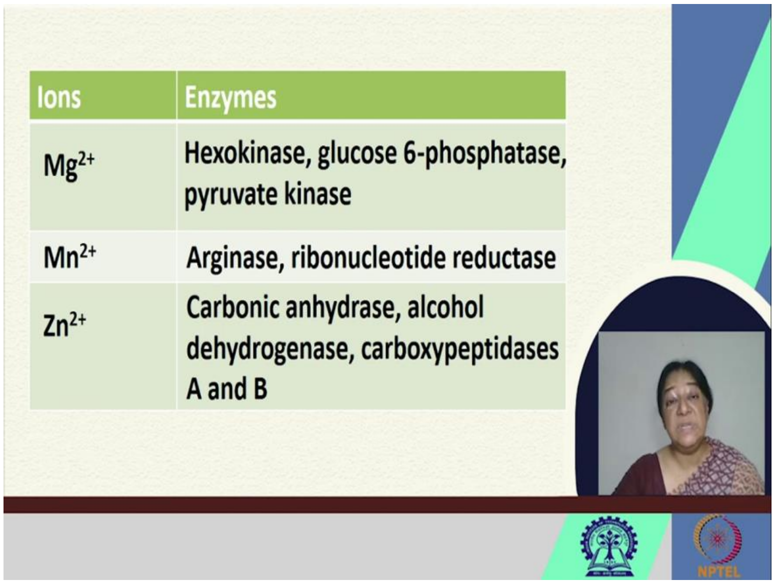

Ions	Enzymes
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

The metal ions present in these different types of enzymes, they have a specific role to play. In addition to the important role that they play in the coordination of specific amino acids with them, in attracting the substrate to the specific active site, is what we will investigate as we go through this module.

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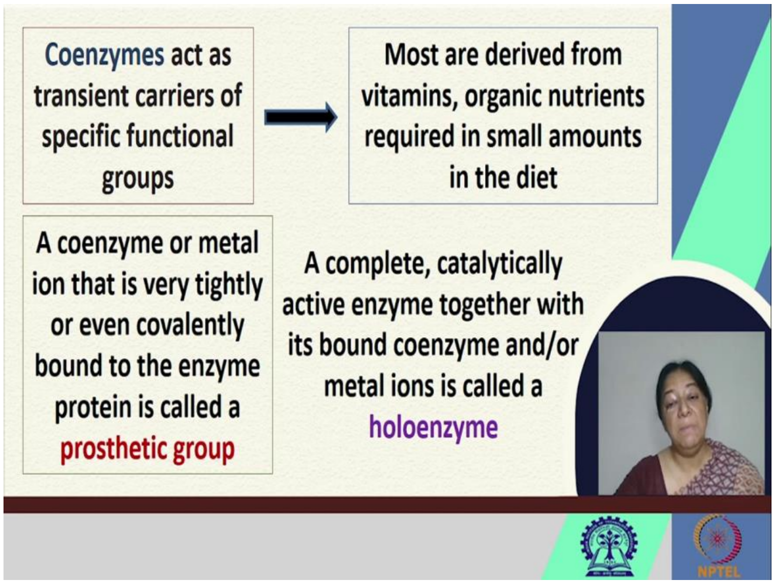

Coenzymes act as transient carriers of specific functional groups

Most are derived from vitamins, organic nutrients required in small amounts in the diet

A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group**

A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme**

These coenzymes, act as the transient carriers of specific functional groups, as a result most are derived from vitamins, organic nutrients and they are required in small amounts in the diet for efficient activity and for efficient functioning. A coenzyme or a metal ion that is very tightly or even sometimes covalently bound to the enzyme is called a prosthetic group.

A complete, catalytically active enzyme, together with its bound coenzyme and / or metal ions is known as a holoenzyme.

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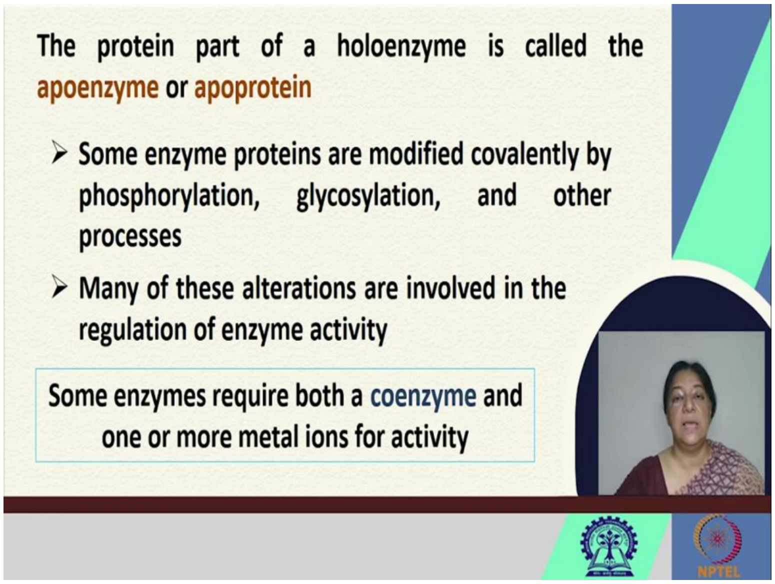

The protein part of a holoenzyme is called the apoenzyme or apoprotein

- Some enzyme proteins are modified covalently by phosphorylation, glycosylation, and other processes
- Many of these alterations are involved in the regulation of enzyme activity

Some enzymes require both a coenzyme and one or more metal ions for activity

The protein part of the holoenzyme is called the apoenzyme or the apoprotein. Some enzyme proteins are also modified covalently by phosphorylation, glycosylation, and other processes, where there would be some other molecule (that is not an amino acid usually), tagged onto the protein. Many of these alterations are involved in the regulation of enzyme activity and are thus essential for their activity. And some enzymes require both a coenzyme and metal enzymes for their activity. We will look at specific examples to see this.

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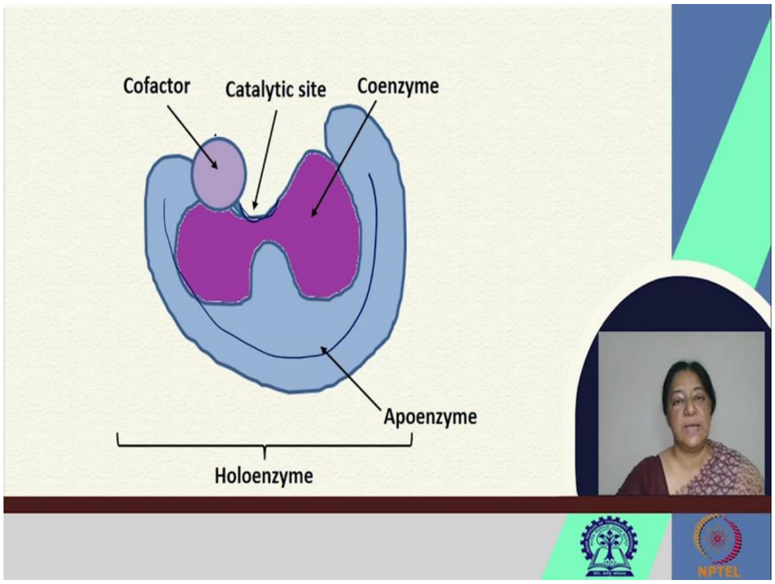

If we look at our cofactor that is bound to our enzyme, what we see is [refer to slide] the major construction of the protein; here we have the active site or the catalytic site where we will have the enzymatic reaction take place; the catalytic reaction to take place. And for the activity we need the cofactor in place.

We need the coenzyme in place and we have the apoenzyme. Together this forms the holoenzyme. And for the enzymatic activity to occur, we need all these parts in place for the proper catalytic activity to occur, for this enzyme.

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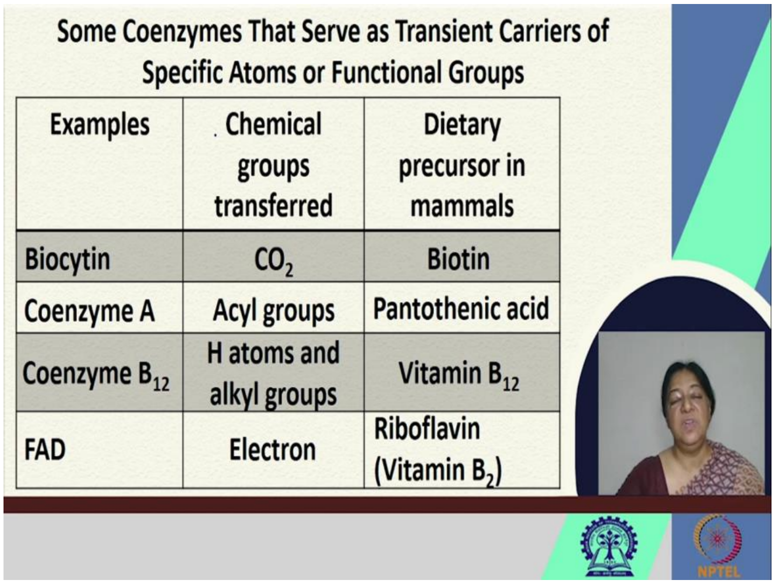

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Examples	Chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid
Coenzyme B_{12}	H atoms and alkyl groups	Vitamin B_{12}
FAD	Electron	Riboflavin (Vitamin B_2)

Some coenzymes that serve as transient carriers of specific atoms of functional groups are biocytin, they transfer carbon dioxide and the dietary precursor in mammals is biotin. Similarly, there are some other examples here. We have coenzyme A where the chemical group transferred are the acyl groups and the dietary precursor is pantothenic acid.

Similarly, we can have coenzyme B_{12}, we have hydrogen atoms or alkyl groups transferred in vitamin B_{12}, which is the dietary precursor. So any reaction that would require the transfer of alkyl groups would need the coenzyme B_{12}, and for the coenzyme B_{12} to be present we have to have vitamin B_{12}. FAD where we have electron transfer, requires riboflavin or vitamin B_2.

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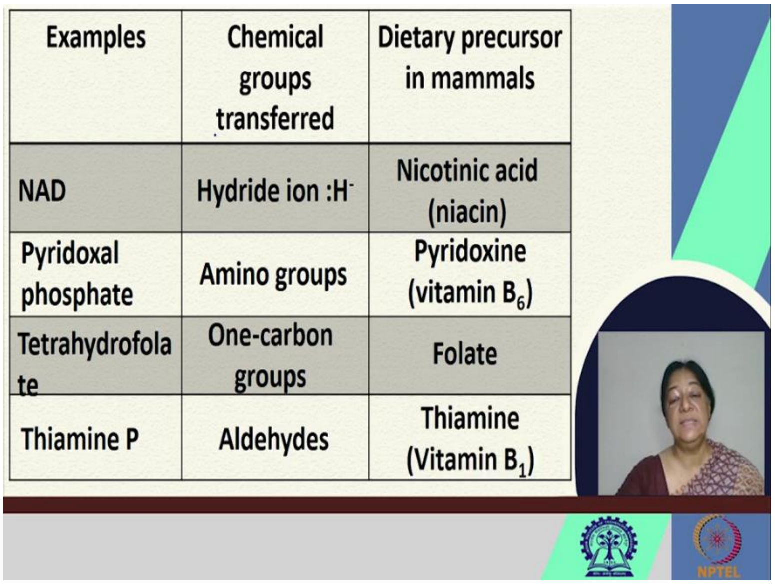

Examples	Chemical groups transferred	Dietary precursor in mammals
NAD	Hydride ion $:H^-$	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine P	Aldehydes	Thiamine (Vitamin B_1)

Some other examples, NAD where we have the transfer of a hydride ion, nicotinic acid in terms of niacin, in dietary precursors. Pyridoxal phosphate, the transfer of amino groups in vitamin B_6. Tetrahydrofolate, one-carbon groups in folate and thiamine P for the transfer of aldehydes in vitamin B_1.

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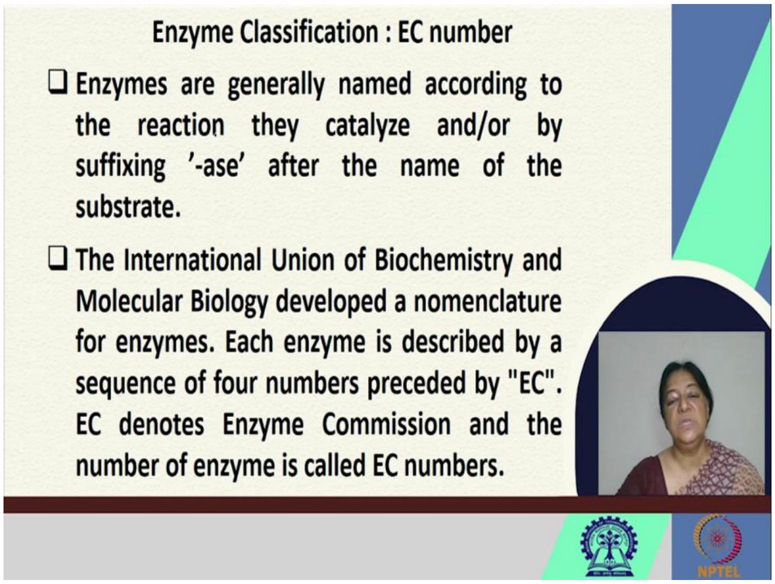

Enzyme Classification : EC number

- ☐ Enzymes are generally named according to the reaction they catalyze and/or by suffixing '-ase' after the name of the substrate.
- ☐ The International Union of Biochemistry and Molecular Biology developed a nomenclature for enzymes. Each enzyme is described by a sequence of four numbers preceded by "EC". EC denotes Enzyme Commission and the number of enzyme is called EC numbers.

If we look at the enzyme classification now, this assists us in understanding what group of enzymes it belongs to. As was mentioned in a couple of slides before, that the enzymes are generally named according to the reaction that they catalyze or by suffixing -ase after the name of the substrate.

The International Union of Biochemistry and Molecular Biology, developed a nomenclature for enzymes. So, each enzyme is described by a sequence of four numbers preceded by 'EC' that denotes the enzyme commission and the number of enzyme is called its EC number.

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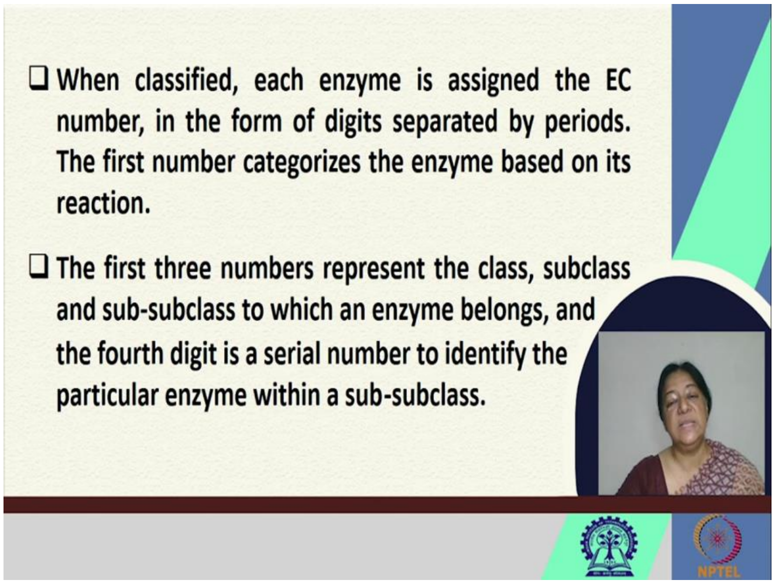

- ☐ When classified, each enzyme is assigned the EC number, in the form of digits separated by periods. The first number categorizes the enzyme based on its reaction.
- ☐ The first three numbers represent the class, subclass and sub-subclass to which an enzyme belongs, and the fourth digit is a serial number to identify the particular enzyme within a sub-subclass.

When classified, each of these enzyme is assigned this EC number in the form of digits separated by periods, much like you would see an IP address and the first number categorizes the enzyme based on its reaction.

The first three numbers represent the class, the subclass and the sub-subclass to which an enzyme belongs, and the fourth digit is a serial number that identifies that particular enzyme within the sub-subclass.

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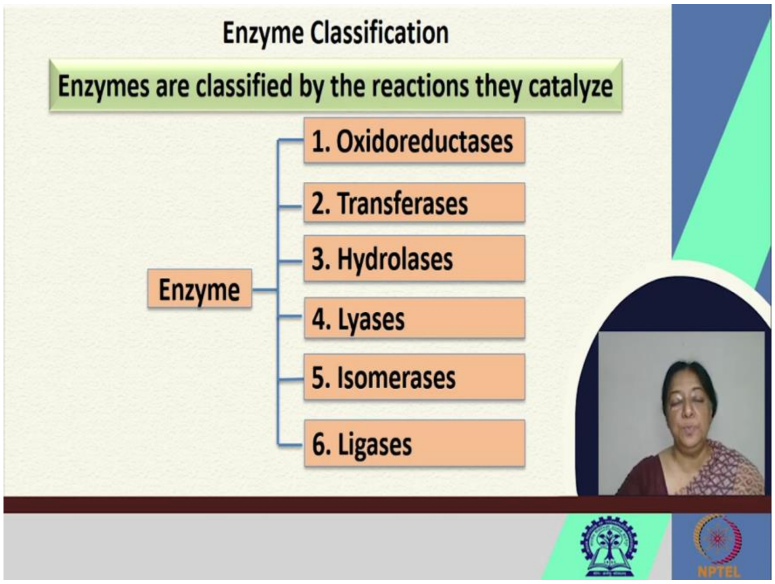

Enzyme Classification

Enzymes are classified by the reactions they catalyze

Enzyme

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

So, if we look at this classification, they are classified by the reactions that they catalyze. If we look at an enzyme there are six enzyme classes; we have the oxidoreductases, the transferases, the hydrolases, the lyases, the isomerases and the ligases. These enzymes are classified accordingly in these six groups of reactions that they catalyze.

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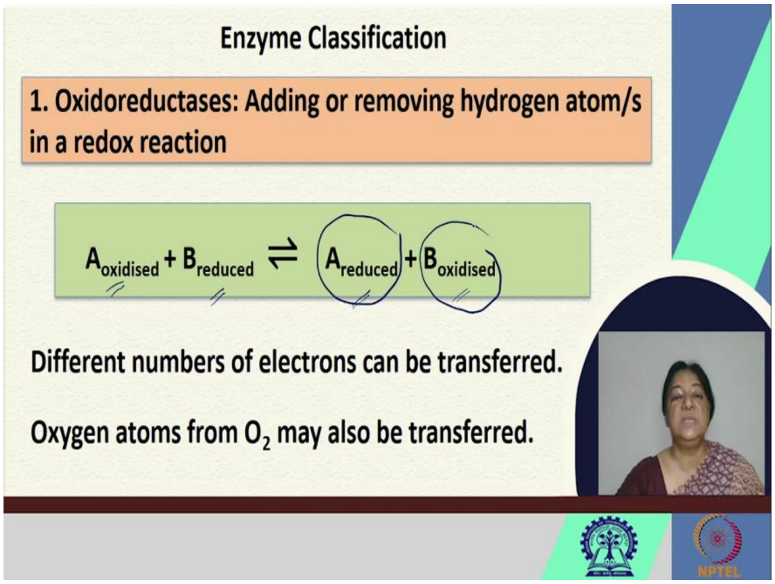

Enzyme Classification

1. Oxidoreductases: Adding or removing hydrogen atom/s in a redox reaction

$$A_{oxidised} + B_{reduced} \rightleftharpoons A_{reduced} + B_{oxidised}$$

Different numbers of electrons can be transferred.

Oxygen atoms from O_2 may also be transferred.

If we look at oxidoreductases; the job of this catalyst is involved in adding or removing hydrogen atoms in a redox reaction. So here [refer to slide] we see oxidized A and reduced B, and because of the transfer or the removal of the hydrogen atom in the redox reaction we have a reversal in the final product where now A is the reduced one and B is the oxidized one.

Different numbers of electrons can be transferred in this type of reaction and oxygen atoms may also be transferred in the set that we would have an oxidation and the oxidizing agent would be reduced.

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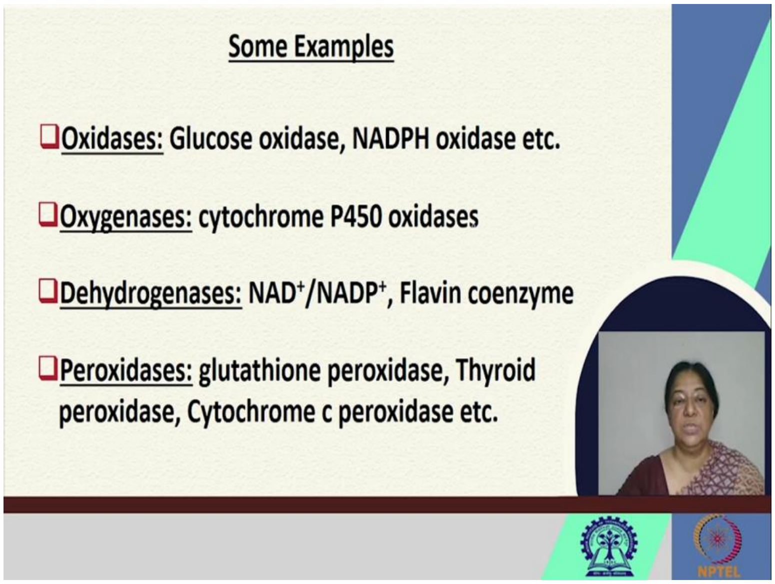

Some Examples

- **Oxidases:** Glucose oxidase, NADPH oxidase etc.
- **Oxygenases:** cytochrome P450 oxidases
- **Dehydrogenases:** NAD$^+$/NADP$^+$, Flavin coenzyme
- **Peroxidases:** glutathione peroxidase, Thyroid peroxidase, Cytochrome c peroxidase etc.

If we have some examples; we have oxidases, glucose oxidase, NADPH oxidase. Cytochrome P450 oxidases is a very important group of enzymes. Dehydrogenases and peroxidases, all of these fall into this category of classification one; oxidoreductases.

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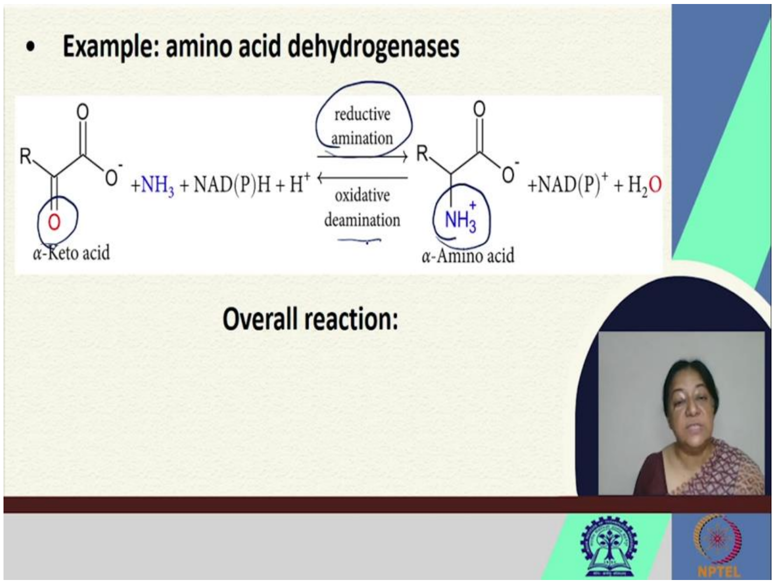

- **Example: amino acid dehydrogenases**

$$\text{α-Keto acid} + NH_3 + NAD(P)H + H^+ \xrightleftharpoons[\text{oxidative deamination}]{\text{reductive amination}} \text{α-Amino acid} + NAD(P)^+ + H_2O$$

Overall reaction:

If we look at an example like amino acid dehydrogenase, the overall reaction results in reductive amination in the forward direction, where we have this [refer to slide] α-keto acid form an α-amino acid.

And the reverse reaction would therefore involve an oxidative deamination to form the ketoacid from the amino acid.

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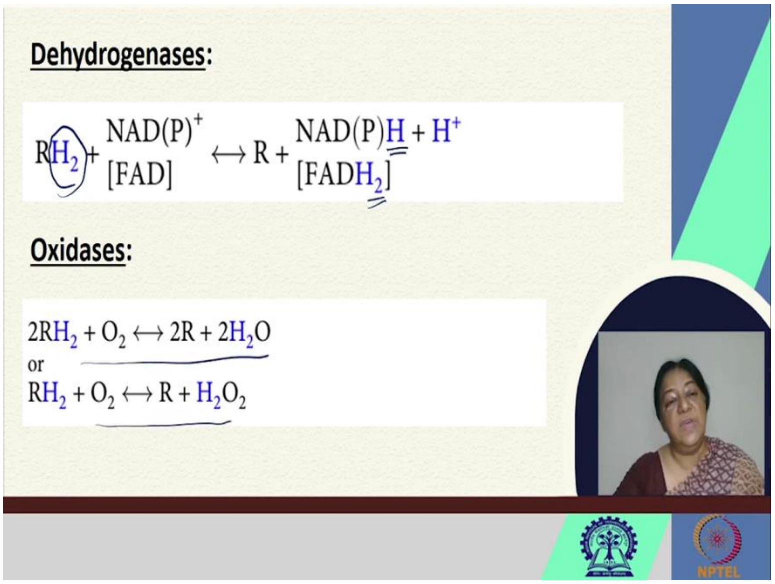

Dehydrogenases:

$$RH_2 + \begin{matrix} NAD(P)^+ \\ [FAD] \end{matrix} \leftrightarrow R + \begin{matrix} NAD(P)H + H^+ \\ [FADH_2] \end{matrix}$$

Oxidases:

$$2RH_2 + O_2 \leftrightarrow 2R + 2H_2O$$

or

$$RH_2 + O_2 \leftrightarrow R + H_2O_2$$

In the case of dehydrogenases, we see a transfer of the hydrogen. In oxidases again we have a similar type of reaction, each of them involving hydrogen or oxygen.

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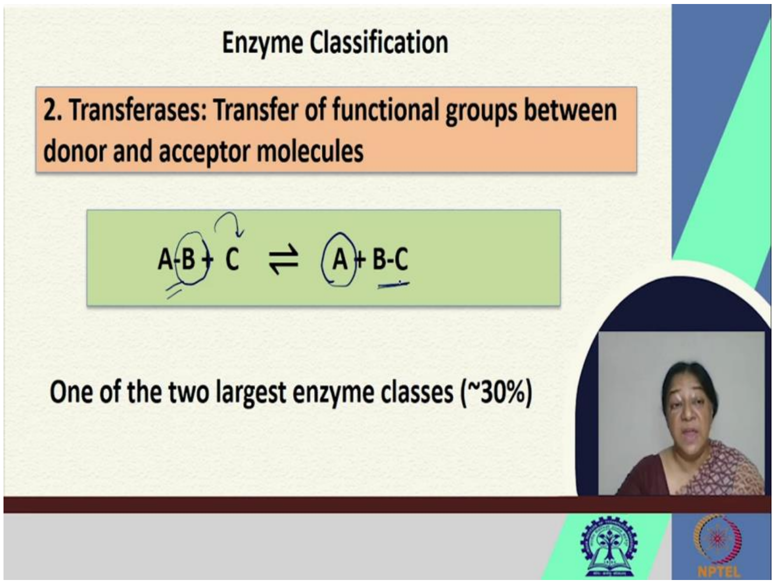

Enzyme Classification

2. Transferases: Transfer of functional groups between donor and acceptor molecules

$A\text{-}B + C \rightleftharpoons A + B\text{-}C$

One of the two largest enzyme classes (~30%)

In the second class where we have the transferases, there is a transfer of functional groups between donor and acceptor molecules. So here [refer to slide] we see A B, where B from A is transferred on to C. So the product is A + B-C where the functional group B has now been transferred to C.

These are one of the two largest enzyme classes and form 30 % of the types of enzymes. A very important transfer, is the transfer of the phosphate group.

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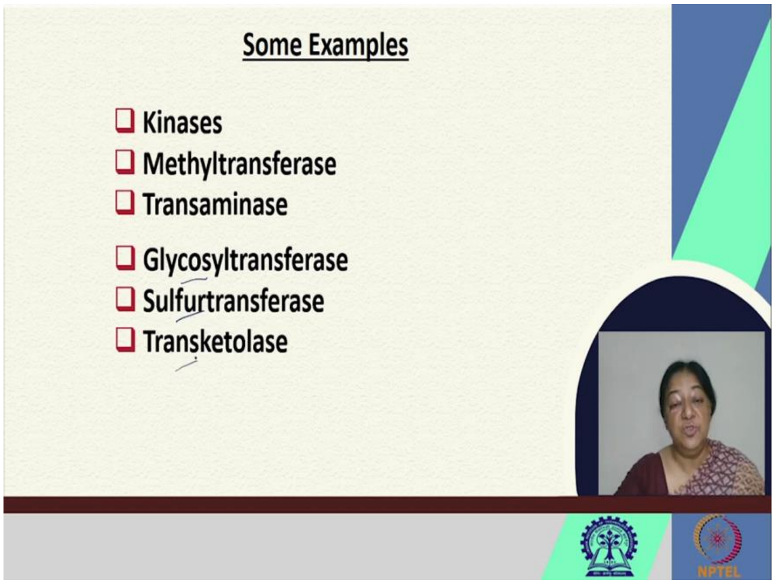

Some Examples

- ☐ Kinases
- ☐ Methyltransferase
- ☐ Transaminase
- ☐ Glycosyltransferase
- ☐ Sulfurtransferase
- ☐ Transketolase

The examples that we see are kinases, methyltransferases, transaminases. Kinase is a specific transferases, that transfer the phosphate group. We also have the other types of transferases, where from the name we can understand what particular group is being transferred and what type of reaction might occur.

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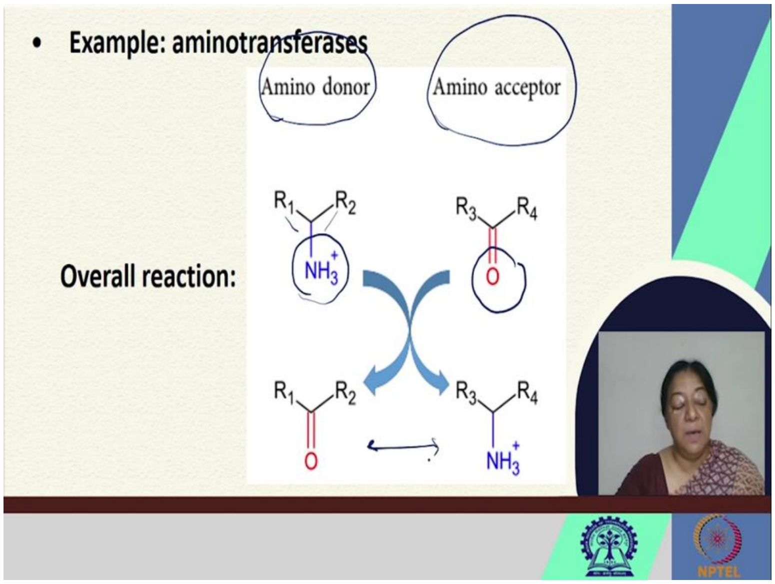

- **Example: aminotransferases**

Amino donor

Amino acceptor

Overall reaction:

If we look at amino transferases, the overall reaction would involve an amino donor that is going to transfer the amine group and an amino acceptor that is going to accept the transferred amino group.

If we have a system where we have R_1, R_2 with the NH_3^+ group here [refer to slide], and we have this where the oxygen atom is, there is an exchange of this where we have the amine donor and the amine acceptor, giving us the overall reaction that involves amino transferase.

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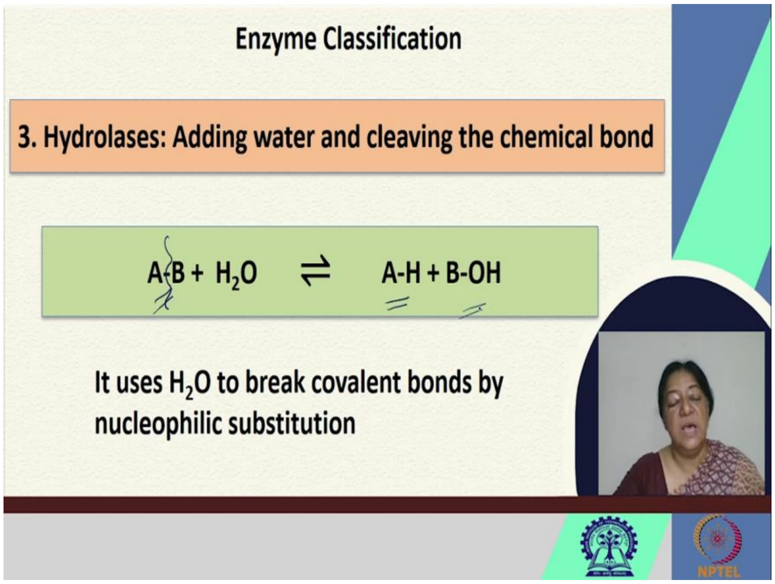

Enzyme Classification

3. Hydrolases: Adding water and cleaving the chemical bond

$$A\text{-}B + H_2O \rightleftharpoons A\text{-}H + B\text{-}OH$$

It uses H_2O to break covalent bonds by nucleophilic substitution

In the third type of enzyme classification, we have hydrolases. In this case there is water being added to the specific bond and there is a cleavage of the chemical bond resulting in A-H + B-OH. This uses water to break covalent bonds, by specific nucleophilic substitution reactions.

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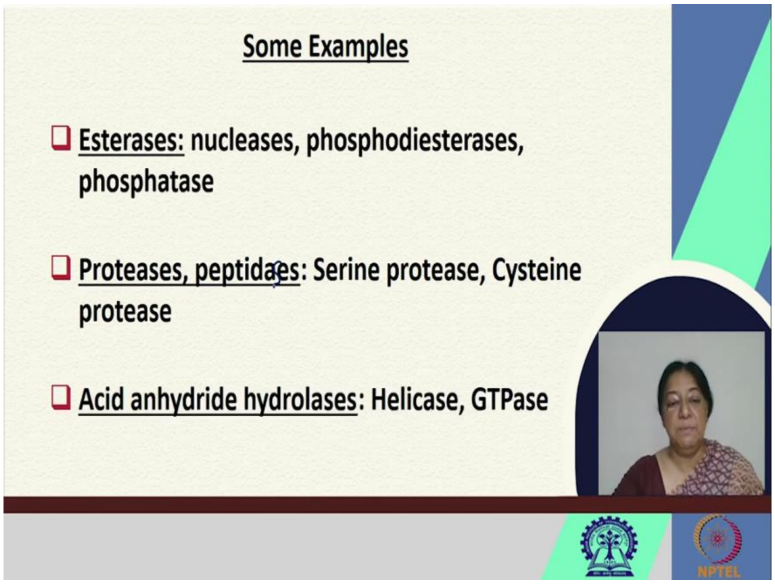

Some Examples

- ☐ **Esterases:** nucleases, phosphodiesterases, phosphatase
- ☐ **Proteases, peptidaes:** Serine protease, Cysteine protease
- ☐ **Acid anhydride hydrolases:** Helicase, GTPase

The examples that we have are esterases, particularly nucleases, phosphatases and proteases involving serine protease and cysteine protease, a peptidaes, that involve the cleavage of a peptide bond; which we will see later when we do enzyme mechanisms.

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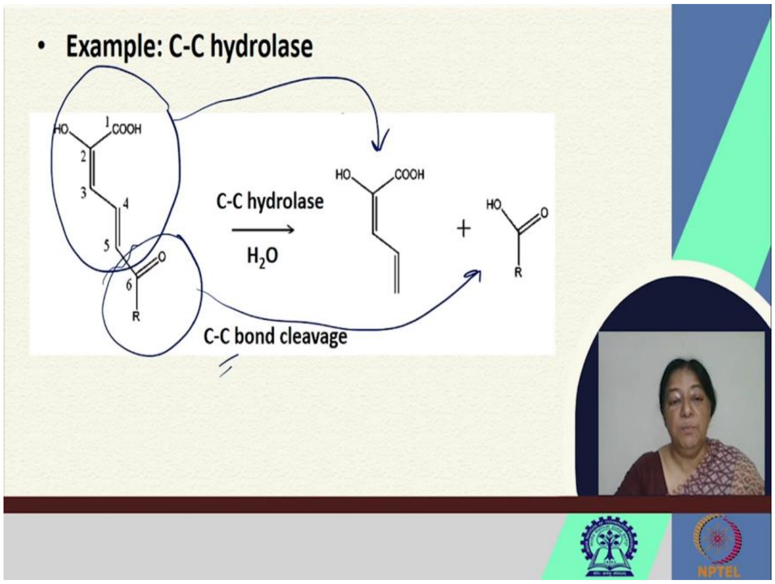

- Example: C-C hydrolase

For example, if we look at a C-C hydrolase, there is a specific reaction where we cleave the C-C bond. We have a cleavage in this [refer to slide] particular reaction, where we have the break here and this part remains as it is and this part has been transferred here. So we have a C-C bond cleavage.

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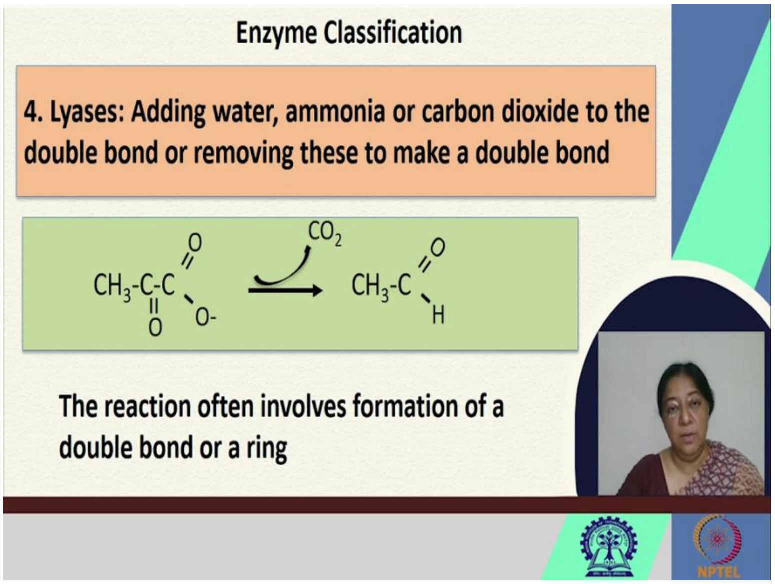

Enzyme Classification

4. Lyases: Adding water, ammonia or carbon dioxide to the double bond or removing these to make a double bond

The reaction often involves formation of a double bond or a ring

In the fourth type of enzyme classification we have lyases. In this case water, ammonia or carbon dioxide is added to a double bond or there is a removal of these small molecules to make a double bond. So the reaction sometimes involves the formation of a double bond or a ring in the structure.

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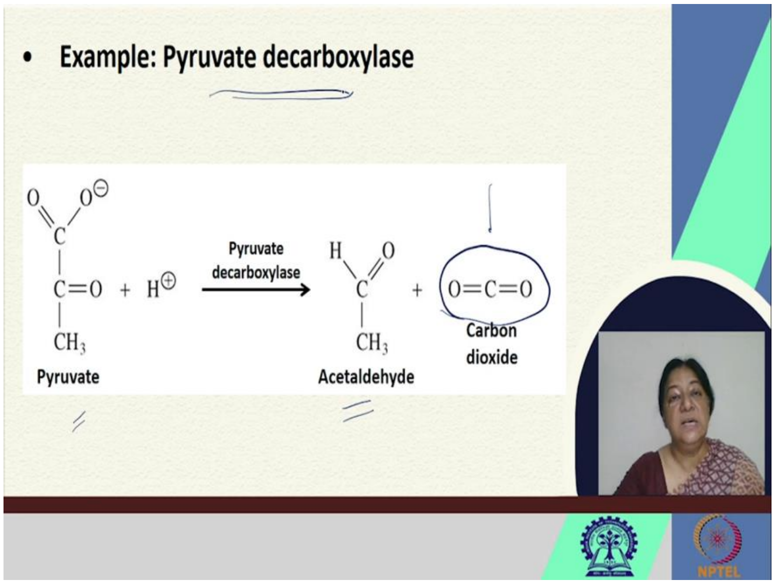

- **Example: Pyruvate decarboxylase**

The examples of such lyases are pyruvate decarboxylase, where we have [refer to slide] the pyruvate molecule lose the carbon dioxide, forming acetaldehyde. So the transfer or the loss of a small molecule, such as ammonia or carbon dioxide would result in a pyruvate decarboxylase, in this case in a specific type of lyase reaction.

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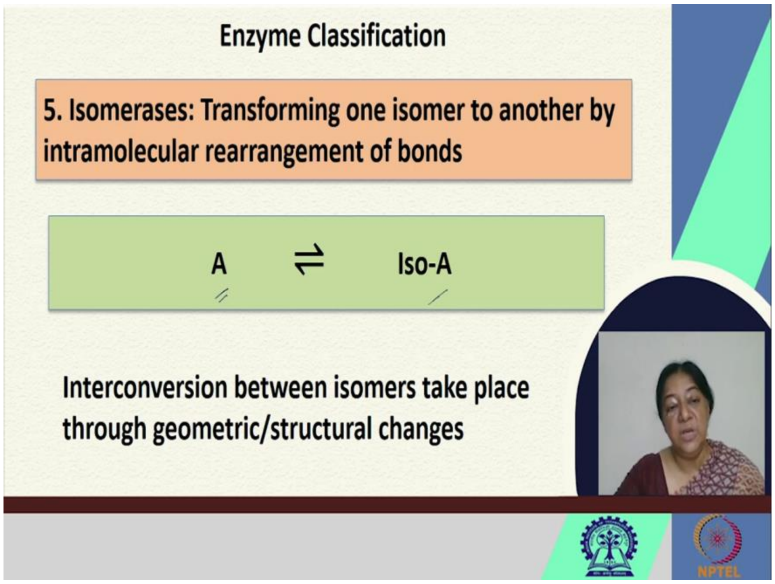

Enzyme Classification

5. Isomerases: Transforming one isomer to another by intramolecular rearrangement of bonds

A ⇌ Iso-A

Interconversion between isomers take place through geometric/structural changes

In the fifth type of enzyme classification, we look at isomerases. As the name implies, this is the transformation of one isomer to another isomer, by intramolecular rearrangement of bonds.

We have A and we have the isomer of A. Now, this is sometimes important in enzyme reactions because it allows the interconversion between isomers that take place through geometric or structural changes.

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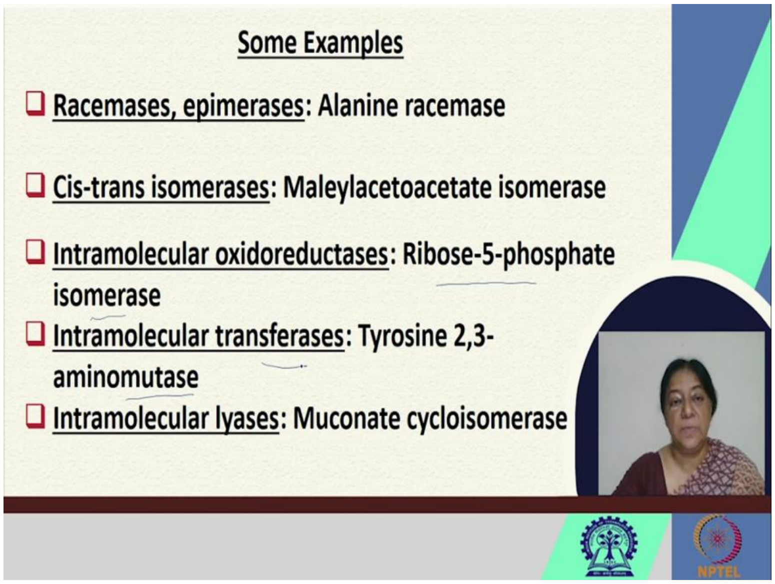

Some Examples

- ☐ Racemases, epimerases: Alanine racemase
- ☐ Cis-trans isomerases: Maleylacetoacetate isomerase
- ☐ Intramolecular oxidoreductases: Ribose-5-phosphate isomerase
- ☐ Intramolecular transferases: Tyrosine 2,3-aminomutase
- ☐ Intramolecular lyases: Muconate cycloisomerase

The specific examples of these types of enzymes are racemases or epimerases as the name implies. We can go from one racemic structure to another using a racemase enzyme or from one epimer to another epimer in another isomerization reaction. One example is alanine racemase. In cis-trans isomerases, we go from a cis configuration to a trans configuration in these types of isomer reactions.

In addition, we can have intramolecular oxidoreductases, where we have a ribose-5-phosphate isomerase or we can have specific mutases, where an intramolecular transferase will take place. In these cases, there is the transfer of a specific group from one position to the other in the same molecule that indicates that it is an isomer of that, but there is a transfer of a moiety from one atom to another atom.

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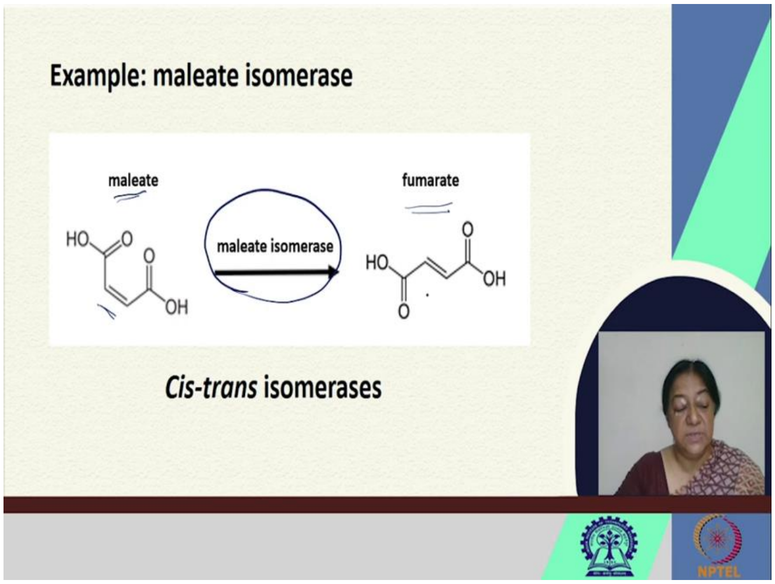

Example: maleate isomerase

Cis-trans **isomerases**

Another example that we can look at is maleate isomerase. In this case we are looking at a cis-trans isomerase. Malic acid and fumaric acid are cis-trans isomers. So when we have the maleate in this case which is the cis isomer, the involvement of the enzyme maleate isomerase is going to be involved in a reaction that would form the isomer of maleate which is fumarate, where we would have the trans configuration.

This is an important reaction in one of the oxidative reactions that are present in our body.

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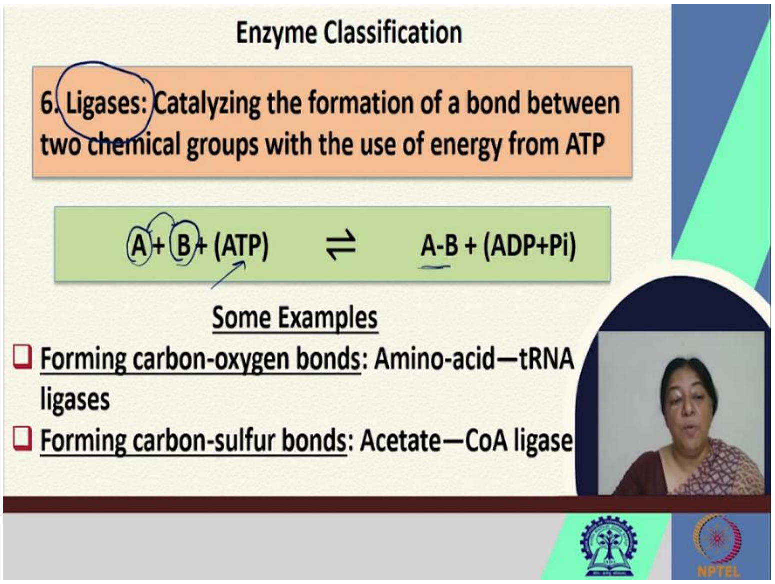

Enzyme Classification

6. Ligases: Catalyzing the formation of a bond between two chemical groups with the use of energy from ATP

$$A + B + (ATP) \rightleftharpoons A\text{-}B + (ADP+Pi)$$

Some Examples

- ☐ Forming carbon-oxygen bonds: Amino-acid—tRNA ligases
- ☐ Forming carbon-sulfur bonds: Acetate—CoA ligase

In this type of enzyme classification in ligases, we have the catalysis where there is the formation of a bond between two chemical groups with the use of energy from ATP.

In this case what happens is we have A, we have B and these two are going to be connected by a bond formation in a ligation reaction, that is going to be due to the presence of the enzyme like some ligase. That will result in the connection of A and B in a covalent bond formation, but with the use of energy from the cleavage of the high energy phosphate bond of ATP.

These are very important reactions because we are forming new types of compounds. For example, we can form carbon-oxygen bonds and form carbon-sulfur bonds. In this case, the enzyme that is used to form the carbon-oxygen bond could be amino acid tRNA ligases and the enzyme that is used to form the carbon-sulfur bond is acetate CoA ligases.

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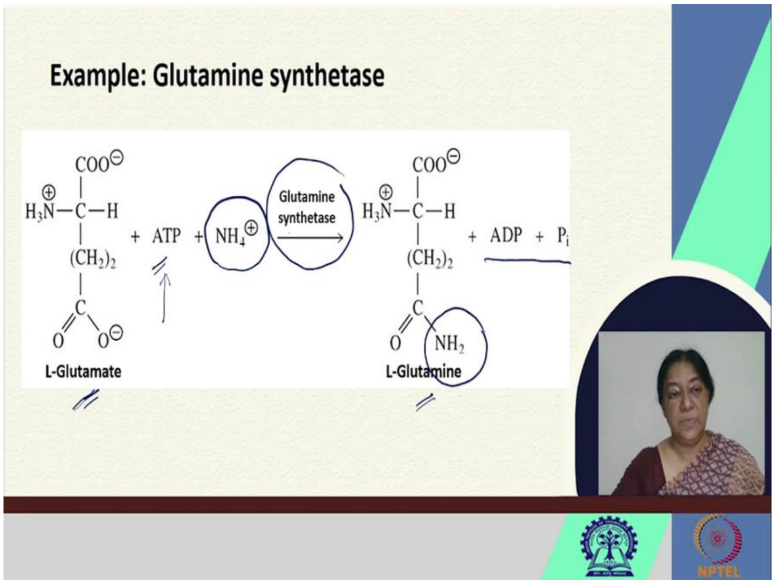

Example: Glutamine synthetase

In a specific example of glutamine synthetase, the synthetase means that it is being formed. In this [refer to slide] case what we see is we have L-glutamate, that is going to form L-glutamine. Glutamine synthetase as the name implies, is the synthesis of glutamine. Now, this particular formation involves the formation of the amide form of glutamic acid. This we know is the amino acid, glutamic acid; the acidic amino acid.

It is going to be ligased with NH_4^+ and we need energy for the reaction. This energy comes from the cleavage of ATP to ADP + Pi, which will involve the ligase or the linking of the amine group or formation of the amide of glutamate, that is glutamine.

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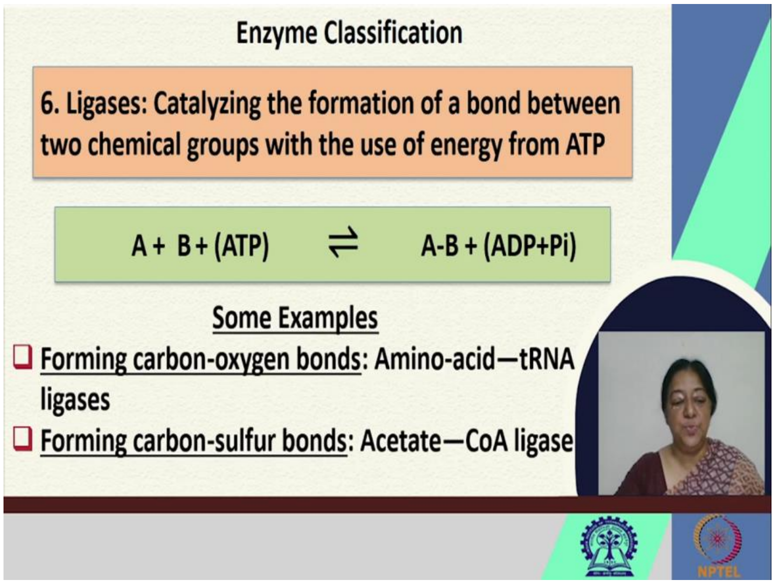

Enzyme Classification

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Some Examples

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☐ Forming carbon-sulfur bonds: Acetate—CoA ligase

In the sixth enzyme classification of ligases, again we look at this [refer to slide] specific bond as we spoke about.

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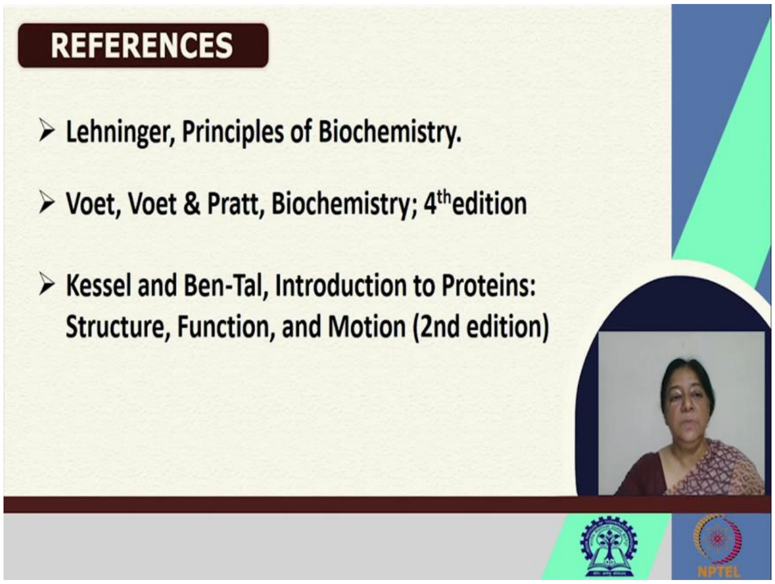

REFERENCES

- Lehninger, Principles of Biochemistry.
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- Kessel and Ben-Tal, Introduction to Proteins: Structure, Function, and Motion (2nd edition)

These [refer to slide] are the references for this particular lecture.

Thank you.