

**Fundamentals of Protein Chemistry**  
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**Module - 12**  
**Special Topics in Protein Chemistry**  
**Lecture - 56**  
**Oxidative Stress in Proteins**

In our final module of the course on fundamentals of protein chemistry, there are special topics in protein chemistry that are going to be discussed. We will start off with oxidative stress in proteins, this will be followed by a special lecture on enzymatic cleavage, followed by intrinsically disordered proteins and then viral proteins and in the last class we will give an overview of the topics that have been covered in the course.

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**CONCEPTS COVERED**

- Oxidative Stress
- Protein oxidation and its outcomes
- Amino acid modifications
- Diseases caused due to oxidative stress
- Antioxidants and their role

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

In this lecture we will look at the concepts covered in terms of oxidative stress, protein oxidation, what we mean by protein oxidation, specific amino acid modifications, the diseases that are

caused due to oxidative stress specific antioxidants and their role to combat oxidative stress and the damages that they cause.

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**KEYWORDS**

- Oxidative stress
- Amino acid modifications
- Dityrosine
- Antioxidants
- Polyphenols

The slide features a list of keywords under a dark red header. A small video inset of the presenter is visible in the bottom right corner. Logos for IIT Bombay and NPTEL are at the bottom.

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**Protein activities**

DNA → **Transcription** (RNA Polymerase) → mRNA → **Translation** (Ribosome) → Primary structure Polypeptide Chain → **Folding and PTM** → Functional Proteins

Peptide bond  
COOH  
H

Primary structure Polypeptide Chain  
● = Amino Acids

Proteins have specific **Structure Function** → **Modifications** → **Biological disorders**

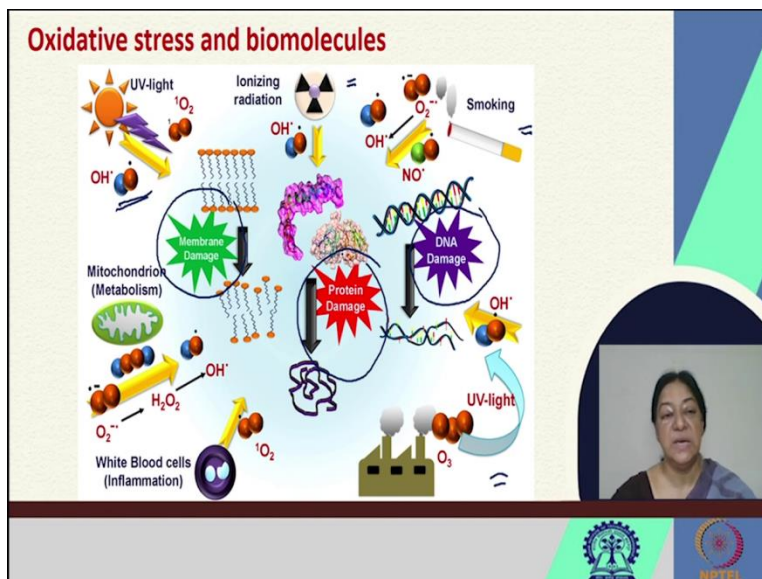
The diagram illustrates the flow from DNA to functional proteins. It includes a chemical structure of a peptide bond and a legend for amino acids. A yellow arrow points from 'Proteins have specific Structure Function' to 'Biological disorders'. A video inset of the presenter is in the bottom right. Logos for IIT Bombay and NPTEL are at the bottom.

These [refer to slide] are specific keywords that we will be looking at as the lecture is delivered. When we look at proteins, we know the central dogma of biology from DNA to RNA to protein. Following this after folding, there is post translational modification and folding may lead to specific types of functional proteins; they could be enzymes, they could be structural proteins as we have seen during the course.

In addition to that there may be post translational modifications again, for specific functions that are required. For the chaperone proteins that we studied in a previous lecture, we know how that can assist in an improper folding process, to get the protein to fold properly.

So in our discussion here, when we try and understand the protein activities, we know that the proteins have a specific structure and associated with that, a specific function. Now modifications of the structure in any manner, can give biological disorders.

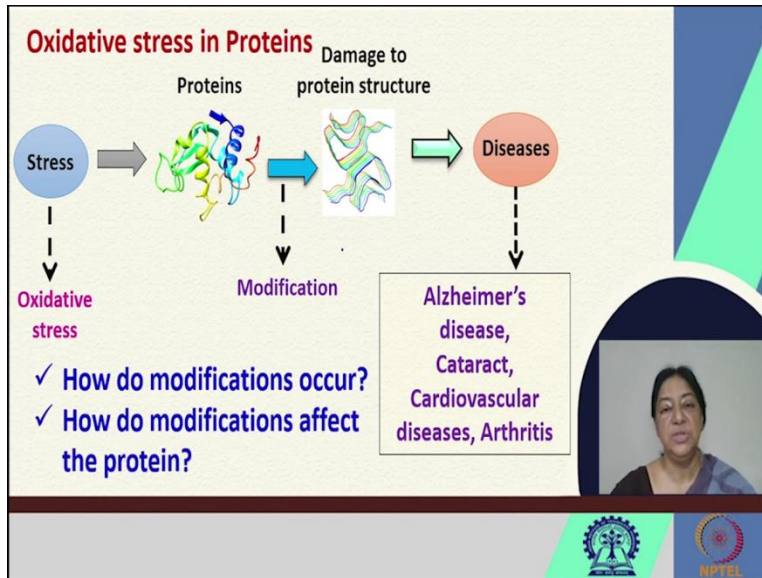
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If we look at oxidative stress and biomolecules in general, there are several aspects that can affect biological macromolecules. So whether we are looking at variations in terms of UV light, ionizing radiation, smoking, UV light from other sources; any such sources can give rise to DNA damage, membrane damage and most importantly in this case, protein damage.

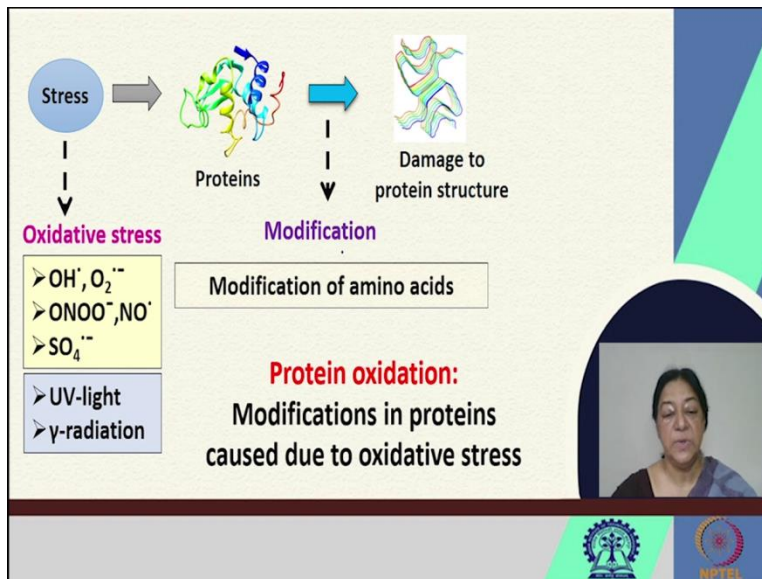
This protein damage can occur in a manner that is going to disrupt the structure and in turn disrupt the function of the protein. So it will not be able to perform the specific activity it has been designed to do.

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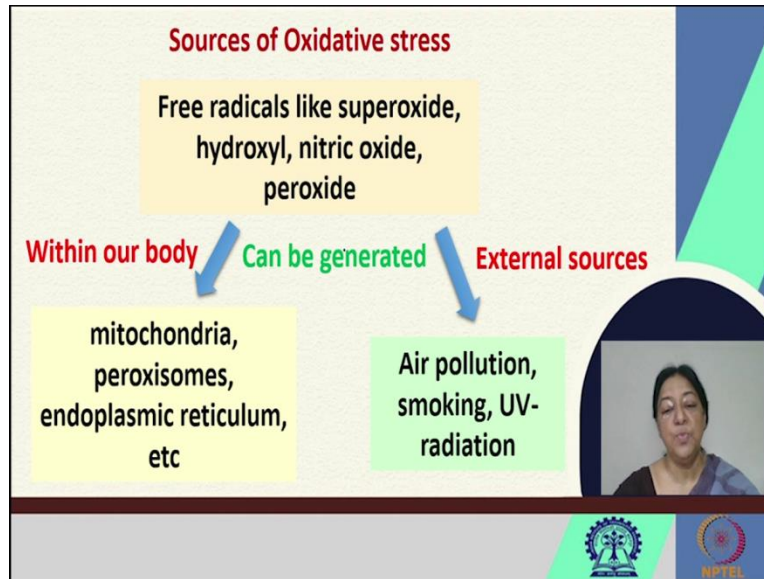
If we look at oxidative stress in proteins, we will understand what we mean by oxidative stress. There is damage to the protein structure, this oxidative stress leads to modification and this leads to disease; diseases such as Alzheimer's disease, cataract, cardiovascular diseases, arthritis and so on. The question is how do these modifications occur and how do the modifications affect the protein?

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If we look at the stress and the proteins and the damage to the protein structure, the oxidative stress can be due to the presence of UV light gamma radiation and is usually caused by several radical formations, as has been shown here [refer to slide]. The modifications that can occur due to the specific amino acid variations, depend upon the type of amino acid the type of protein and its structural components.

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The modifications in proteins can occur at specific amino acids, which we will be looking at in a moment. The sources of oxidative stress are free radicals such as superoxide, hydroxyl, nitric oxide, peroxide and these can be generated within our body due to specific biochemical reactions that occur in mitochondria, peroxisomes, endoplasmic reticulum and can also be generated from external sources such as air pollution, smoking and UV radiation.

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**Reactive oxygen species (ROS)**

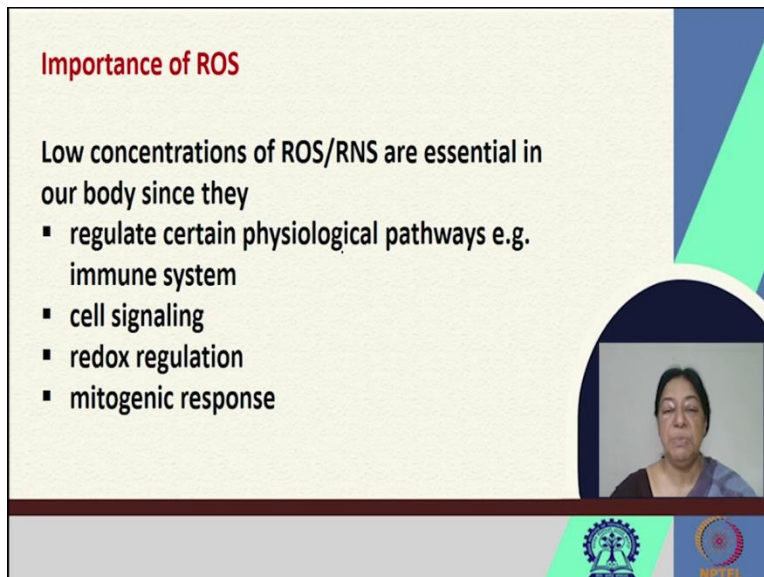
- They are highly reactive chemicals formed from  $O_2$
- E.g.: peroxides, superoxide, hydroxyl radical, singlet oxygen
- Reduction of molecular oxygen during aerobic respiration produces superoxide, a precursor to most other reactive oxygen species

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

The reactive oxygen species that is formed, are highly reactive chemicals that are formed from oxygen. For example like was mentioned peroxide, superoxide, hydroxyl radical and singlet oxygen. The reduction of molecular oxygen during aerobic respiration, produces superoxide.

This superoxide is a precursor to most other reactive oxygen species and these are what cause damage.

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**Importance of ROS**

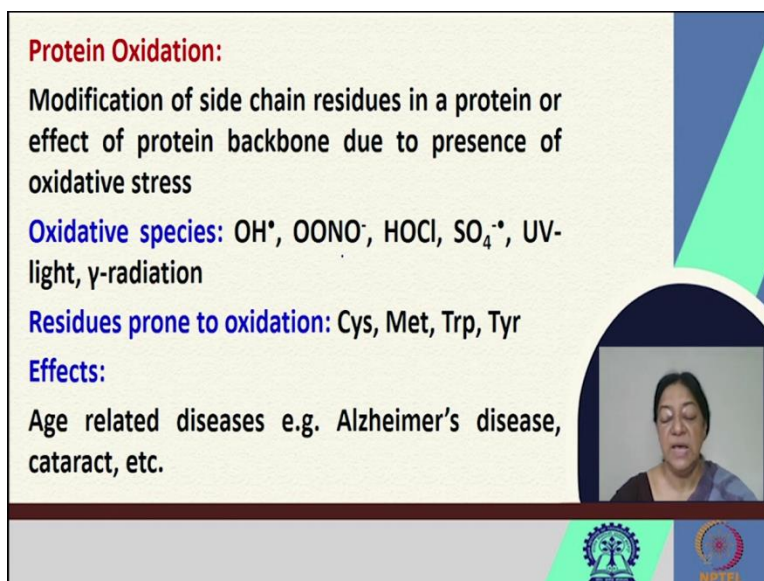
Low concentrations of ROS/RNS are essential in our body since they

- regulate certain physiological pathways e.g. immune system
- cell signaling
- redox regulation
- mitogenic response

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 NPTEL logo on the right.

However, low concentrations of these ROS, the reactive oxygen species or the RNS, that is the reactive nitrogen species are essential in our body since they have certain functions in terms of a regulation of certain physiologic pathways for example, in the immune system, for cell signaling, for redox regulation and for mitogenic responses.

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**Protein Oxidation:**

Modification of side chain residues in a protein or effect of protein backbone due to presence of oxidative stress

**Oxidative species:**  $\text{OH}^\bullet$ ,  $\text{OONO}^\bullet$ ,  $\text{HOCl}$ ,  $\text{SO}_4^{\bullet-}$ , UV-light,  $\gamma$ -radiation

**Residues prone to oxidation:** Cys, Met, Trp, Tyr

**Effects:**

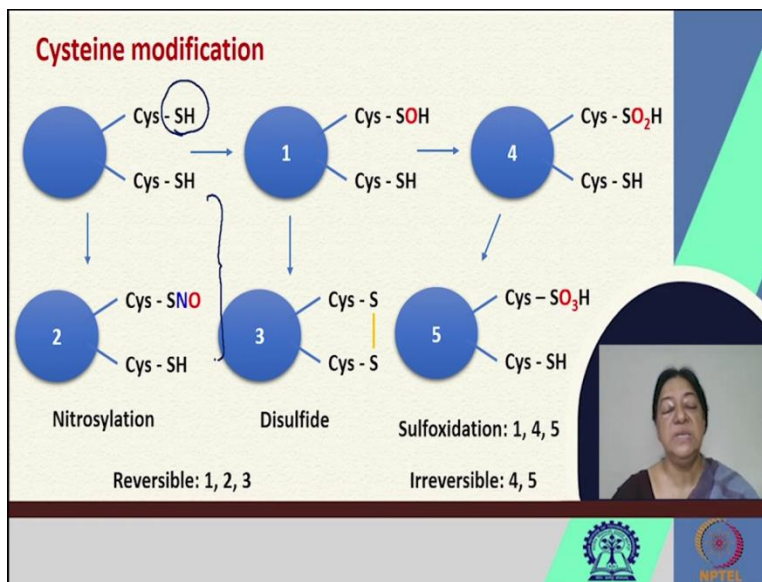
Age related diseases e.g. Alzheimer's disease, cataract, etc.

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The protein oxidation therefore that occurs, is a modification of a side chain residue in a protein that can affect the protein backbone due to the presence of oxidative stress in the terms of the

components that we looked at, whether they are ROS or RNS. So the oxidative species are these and the residues prone to oxidation mostly are cysteine, methionine, tryptophan and tyrosine. The effects are age related diseases as was mentioned.

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If we look at cysteine modification, we want to see what are the modifications that are possible in these residues. For cysteines we know that we have the sulfur containing side chain; this can form disulfide bonds. Now we realize the formation of the disulfide bond or the reduction to the cysteine, is a redox type of reaction where we have specific components possible, specific variations possible, due to this nitrosylation, due to oxidation, due to disulfide formation, due to other types of components.

In nitrosylation, sulfoxidation and disulfide formation some of these specific reactions are reversible in nature. However, some of them are irreversible resulting in permanent damage to the cysteine residues in terms of its functionality; in terms of its ability to form disulfide linkages.

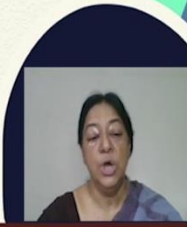

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**Thiol oxidation**

Oxidation of cysteines is a widely recognized mechanism of redox signaling.

Currently, there is an interest in oxidation of thiols which results in the addition of oxygen(s) to the sulfur group of the amino acid cysteine.

The reaction of hydrogen peroxide ( $H_2O_2$ ) with the deprotonated cysteinyl thiol of proteins produces an oxidized thiol or sulfenic acid (R-SOH).



So thiol oxidation, that is an oxidation of the cysteines, is a widely recognized mechanism of redox signaling as is understood from the variations possible. There is interest of the thiols which results in the addition of oxygens to the sulfur group of the amino acid cysteine. The reaction for example of hydrogen peroxide with the deprotonated cysteinyl thiol of proteins, produces an oxidized thiol or sulfenic acid.

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**Thiol oxidation**

- Sulfenic acid may be oxidized again to yield a hyperoxidized sulfenic acid cysteine (R-SO<sub>2</sub>H).  
 $R-SOH \rightarrow R-SO_2H \rightarrow R-SO_3H$
- With increasing levels of reactive species, cysteines can further be oxidized to a sulfonic acid (R-SO<sub>3</sub>H).

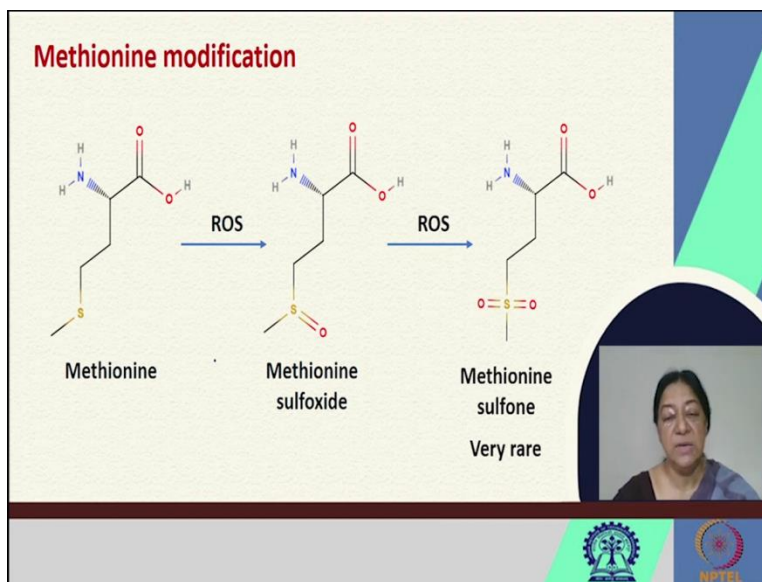
- Sulfenic state can only be reversed enzymatically
- Sulfonic acid modification is considered irreversible -represents protein damage

There are different possibilities that can occur in thiol oxidation. Sulfenic acid for example, may be oxidized again to yield a hyper oxidized sulfenic acid cysteine R-SO<sub>2</sub>H. This in turn with increasing levels of reactive species, they can be further oxidized to what is called sulfonic acid R-SO<sub>3</sub>H. So sulfenic state can be reversed enzymatically, but the sulfonic acid modification is considered irreversible and this results in protein damage.

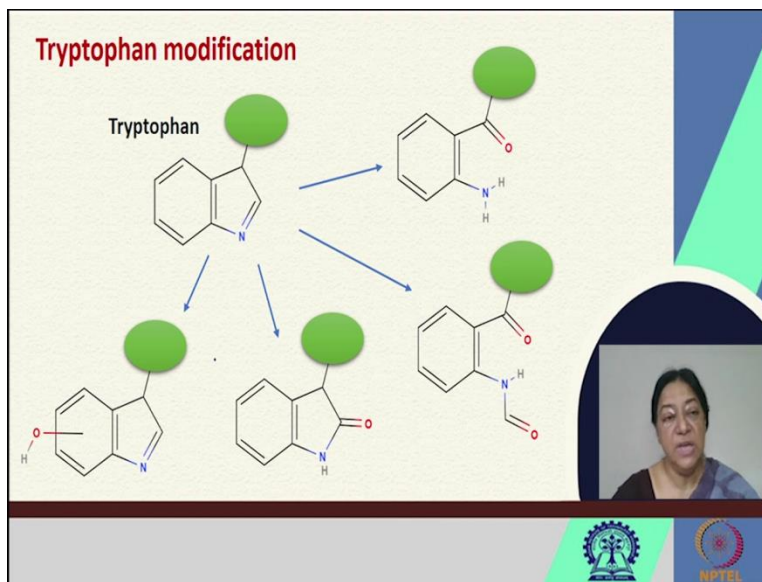


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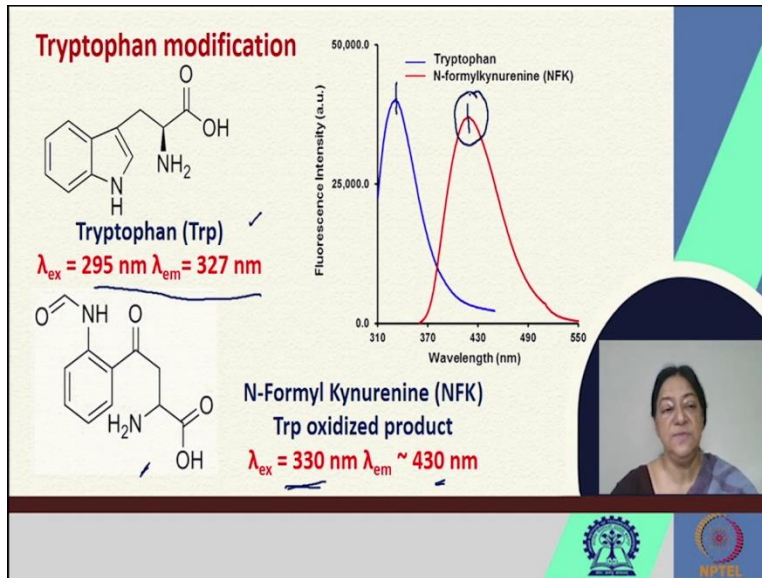
Methionine modification is also possible due to the presence of ROS, where we get methionine sulfoxide and further ROS can give me thionine sulfone, but the sulfoxide part is more common.

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In tryptophan modification, we know that the tryptophan has an indole moiety as its side chain. Tryptophan modifications can occur due to the presence of the reactive oxygen species, giving variations in their structure and a disruption of the indole ring.

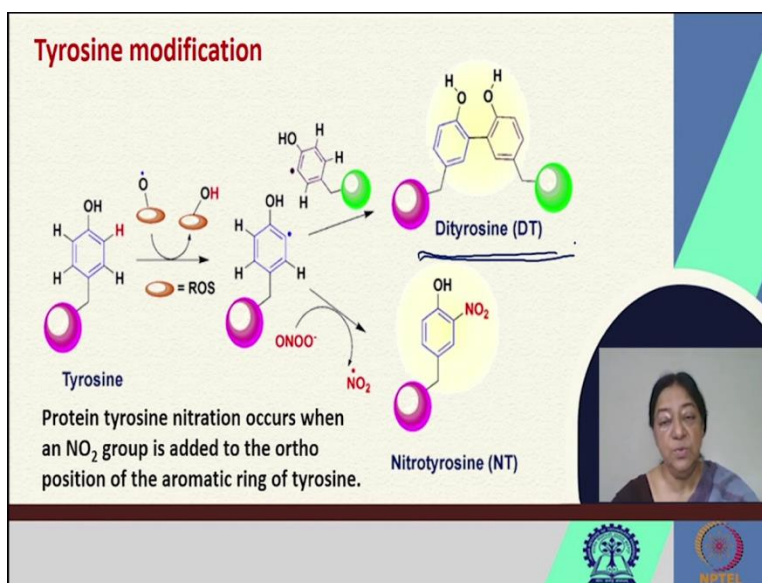
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The tryptophan modifications can give a specific product known as N-Formyl Kynurenine, NFK. This is a tryptophan oxidized product which has the structure shown [refer to slide] and it has specific spectral characteristics for example, as in tryptophan we know the excitation can be a 295 nm with an emission around 330 nm, depending upon the conditions. In this case the excitation of NFK is around 330 nm and the emission is around 430 nm.

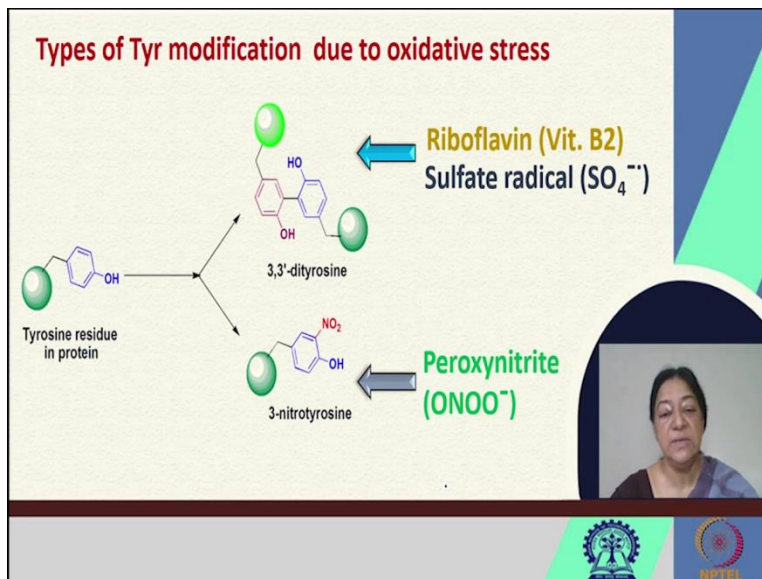
What this means is that if we have the presence of NFK in a species due to the tryptophan modification, it can be easily monitored by fluorescence spectroscopy. This gives us an indication or gives us an idea of what is called a biomarker, telling us that if NFK is present it means that the tryptophan has been modified and this can be tested for several modifications in proteins due to oxidative stress.

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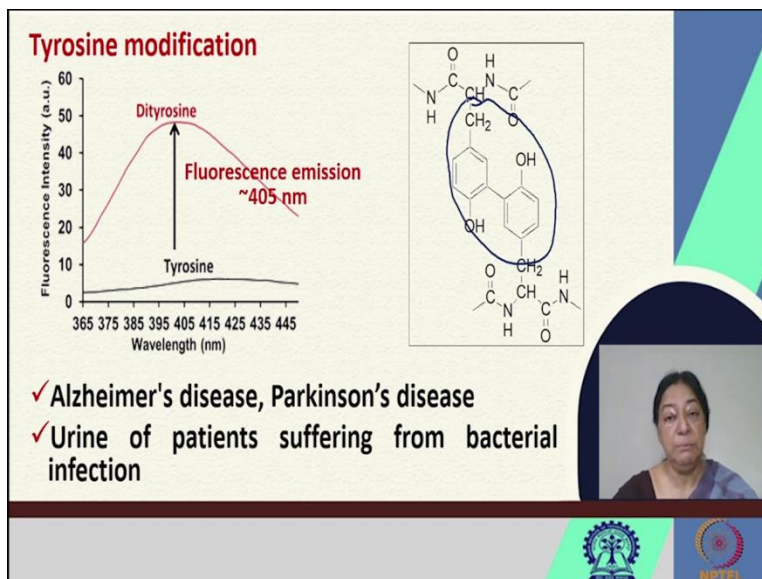
In case of tyrosine modification, there are specific possibilities that can occur. We can have dityrosine formation, where we have a cross linking of two side chains of tyrosine, two tyrosine radicals that can form dityrosine, which is another indicator in addition. There is also the formation of nitrotyrosine where the protein nitrous tyrosine nitration occurs when an  $\text{NO}_2$  group is added to the ortho position of the aromatic ring of tyrosine, the side chain of tyrosine.

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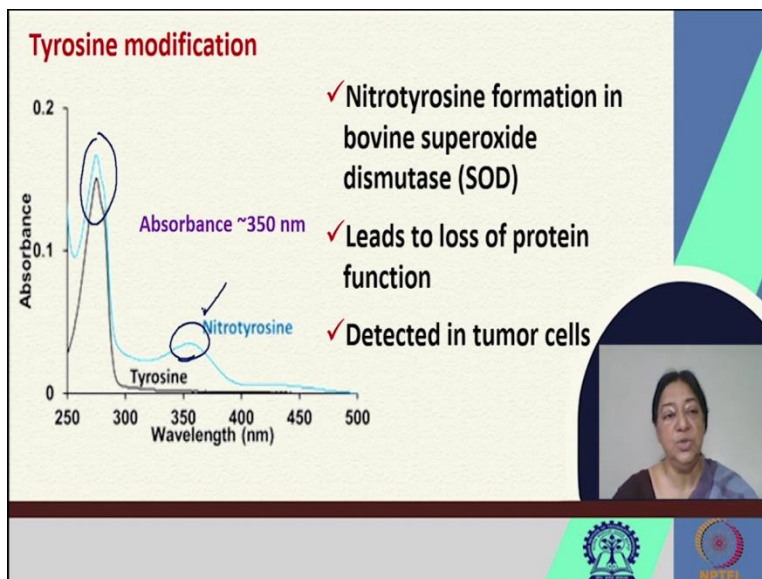
So the types of tyrosine modifications due to oxidative stress, can be in terms of 3, 3 prime dityrosine or 3 nitrotyrosine and these can be achieved in different ways as well.

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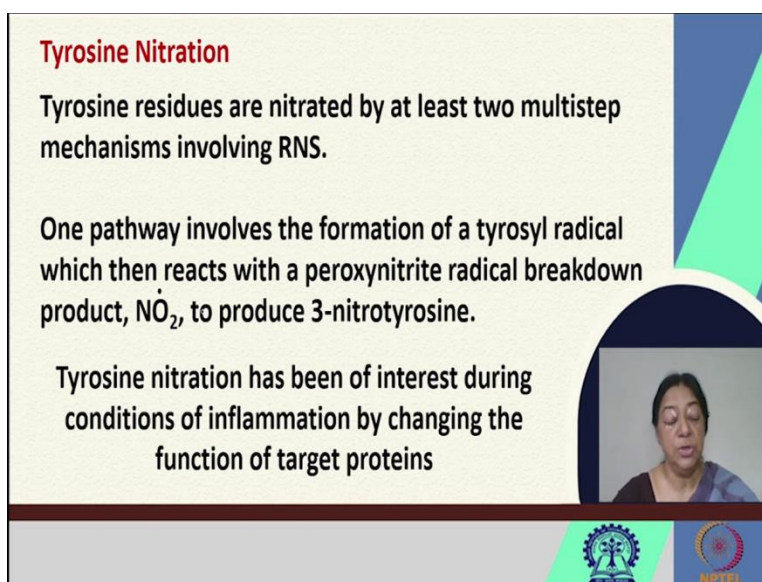
The dityrosine radical gives us a specific fluorescence signal due to the presence of the dityrosine formation and this again can be an indication of oxidative stress. The fluorescence emission is observed in Alzheimer's disease, parkinsons disease and also from the urine of patients suffering from any bacterial infection; so it can be an indicator.

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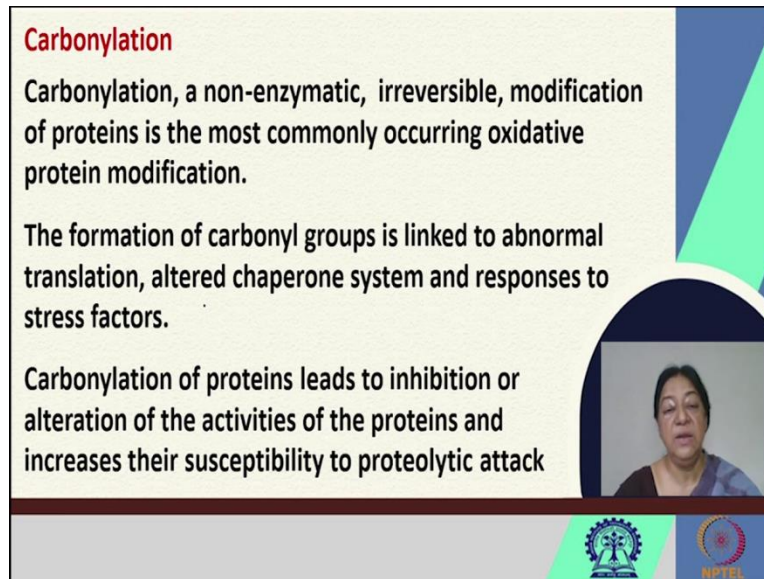
Similarly, we can have the absorbance signal of nitrotyrosine which is distinct from that of tyrosine. We have the signal here also, but this is an additional signal that is observed which can be used. So the nitrotyrosine formation in bovine superoxide dismutase has been seen to lead to loss of protein function and it can also be detected in tumor cells.

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The tyrosine residues are therefore nitrated by at least two multistep mechanisms that involve reactive nitrogen species. One pathway involves the formation of a tyrosine radical, which then reacts with the peroxynitrite radical breakdown product, that is  $\text{NO}_2 \cdot$ , to produce 3-nitrotyrosine. This tyrosine nitration has been of interest during conditions of inflammation, by changing the function of target proteins.

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**Carbonylation**

Carbonylation, a non-enzymatic, irreversible, modification of proteins is the most commonly occurring oxidative protein modification.

The formation of carbonyl groups is linked to abnormal translation, altered chaperone system and responses to stress factors.

Carbonylation of proteins leads to inhibition or alteration of the activities of the proteins and increases their susceptibility to proteolytic attack

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Carbonylation is a nonenzymatic, irreversible modification of proteins and is the most commonly occurring oxidative protein modification. The formation of the carbonyl groups is linked to abnormal translation, altered chaperone system and responses to stress factors. This carbonylation of proteins can lead to inhibition or alteration of the activities of the proteins and also increase their susceptibility to proteolytic attack.

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Carbonyl groups are generated through oxidative cleavage of proteins by an  $\alpha$ -amidation pathway or by formation of peptides with an  $\alpha$ -keto derivatives at the N-terminus

Carbonylation is a metal-catalyzed oxidation (MCO) occurring during the interaction of reduced metal ions, such as  $\text{Fe}^{+2}$  or  $\text{Cu}^+$  with  $\text{H}_2\text{O}_2$

Carbonylated proteins have been found in all plant cell cellular compartments: cytosol, chloroplasts, peroxisomes, nucleus, and mitochondria

These carbonyl groups are generated through an oxidative cleavage of proteins, by what is called an  $\alpha$ -amidation pathway or by the formation of peptides with an  $\alpha$ -keto derivative at the N terminus.




So this is important and carbonylation is a metal catalyzed oxidation, occurring during the interactions of reduced metal ions for example,  $\text{Fe}^{+2}$  or  $\text{Cu}^+$  with  $\text{H}_2\text{O}_2$ . These carbonylated proteins have been found in all plant cell cellular compartments; for the example in cytosol, chloroplasts, peroxisomes, nucleus and also mitochondria.

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**S-glutathionylation**

Glutathionylation of proteins is the modification of a reactive protein cysteine thiol by the reduced glutathione (GSH) and occurs under both normal or oxidative stress conditions.

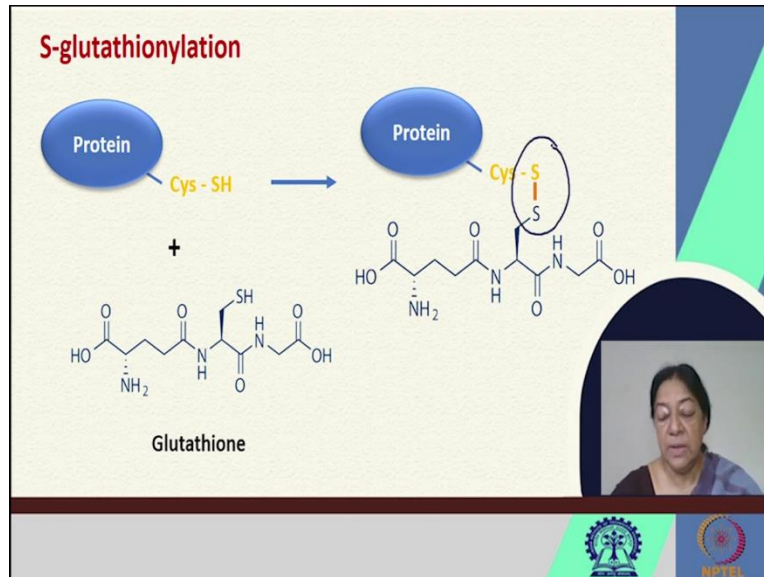
- S-glutathionylation regulates the activity of proteins that contain the active cysteine residue in their structure,
- regulates the oxidoreductive pathway of signal transduction,
- and participates in the regeneration of antioxidant enzymes.

Another change is S-glutathionylation. This is the modification of a reactive protein cysteine thiol by reduced glutathione and occurs both in normal as well as oxidative stress conditions. This S-glutathionylation regulates the activity of proteins, that contain an active cysteine residue

in their structure. They regulate therefore the oxidoreductive pathway of signal transduction and they also participate in the regeneration of antioxidant enzymes.

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So if we have the protein that has this [refer to slide] particular cysteine residue with the free thiol, then this is glutathione that is now going to interact to form this disulfide bond. This then prevents the disulfide bond formation with other cysteine residues, in the formation of what we call a cystine.

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**Glutathionylation is involved in processes such as glycolysis, signal transduction, protein degradation, intracellular trafficking, and protein folding.**

- Non-enzymatic glutathionylation reactions are non-specific and seem to be associated with oxidation stress.
- Enzymatic glutathionylation reactions catalyzed by glutaredoxin in both directions are highly specific, reversible.

It can protect protein thiols from irreversible inactivation but can also alter the activity of many proteins.

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So this is involved in processes such as glycolysis, signal transduction, protein degradation, intracellular trafficking and also protein folding. The non-enzymatic glutathionylation reactions

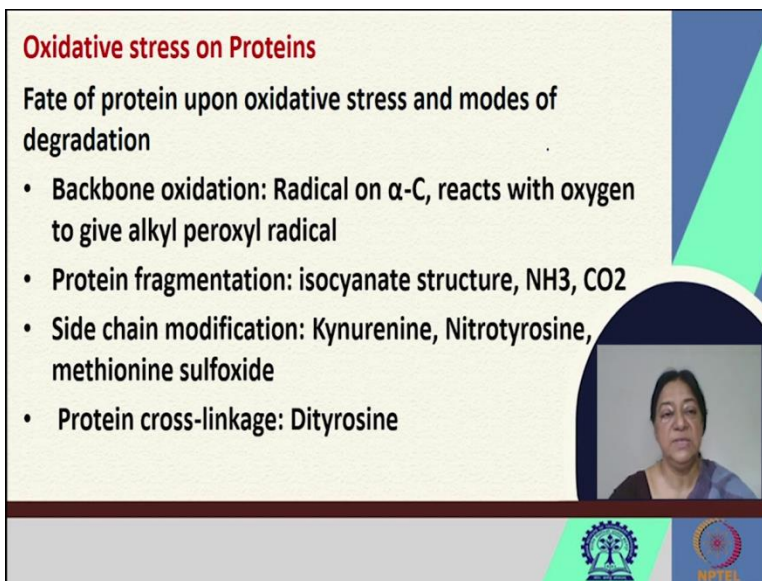
are non specific and they are associated with oxidative stress. The enzymatic reactions are catalyzed by glutaredoxin in both directions, extremely specific and reversible as well. This can protect thiols from irreversible inactivation, but can also alter the activity of many proteins once the adduct is formed.

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**Oxidative stress on Proteins**

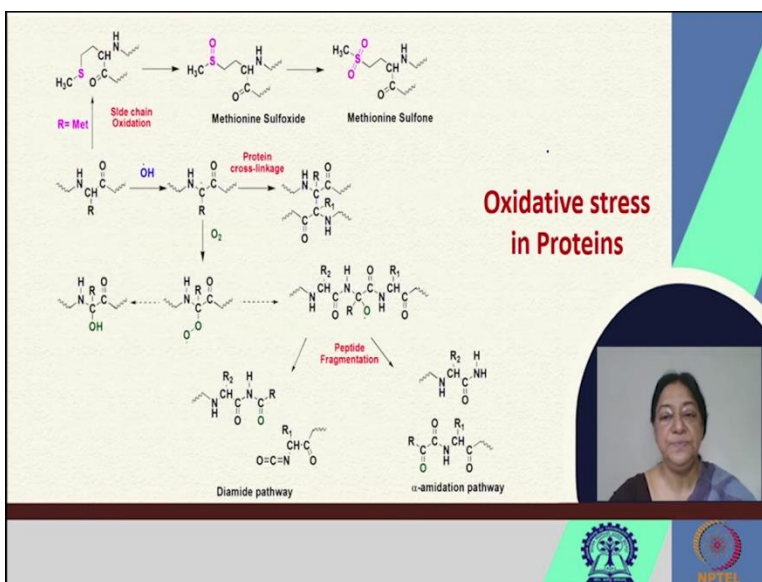
**Fate of protein upon oxidative stress and modes of degradation**

- **Backbone oxidation:** Radical on  $\alpha$ -C, reacts with oxygen to give alkyl peroxy radical
- **Protein fragmentation:** isocyanate structure,  $\text{NH}_3$ ,  $\text{CO}_2$
- **Side chain modification:** Kynurenine, Nitrotyrosine, methionine sulfoxide
- **Protein cross-linkage:** Dityrosine



If we look at oxidative stress on proteins, there is a specific fate of the protein upon oxidative stress and there are modes of degradation. There may be backbone oxidation, where a radical could act on an  $\alpha$ -C, react with oxygen to give an alkyl peroxide radical; protein fragmentation may occur; side chain modifications as we saw in terms of tryptophan, tyrosine, methionine and cysteine; and protein cross linkages in terms of the cross linking, based on two tyrosine radicals.

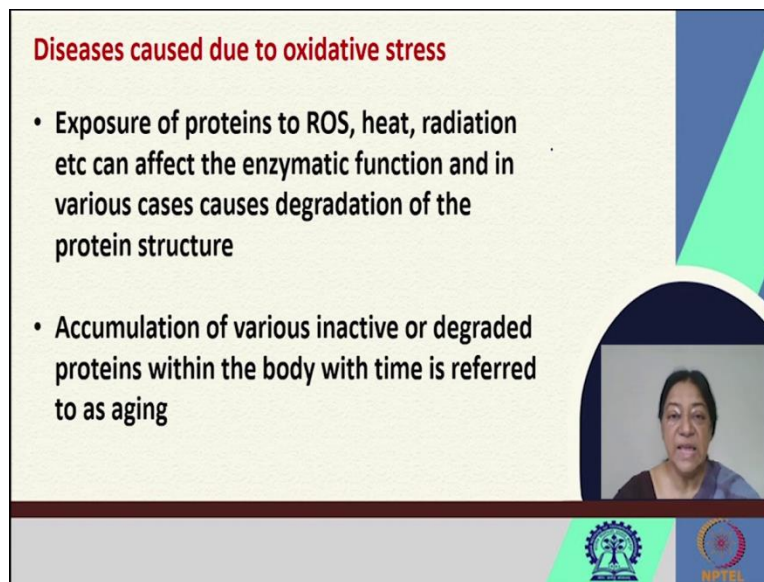
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So oxidative stress in proteins can be summarized here [refer to slide], where we have an ammunition pathway, peptide fragmentation, protein cross linking and other side chain oxidative processes.

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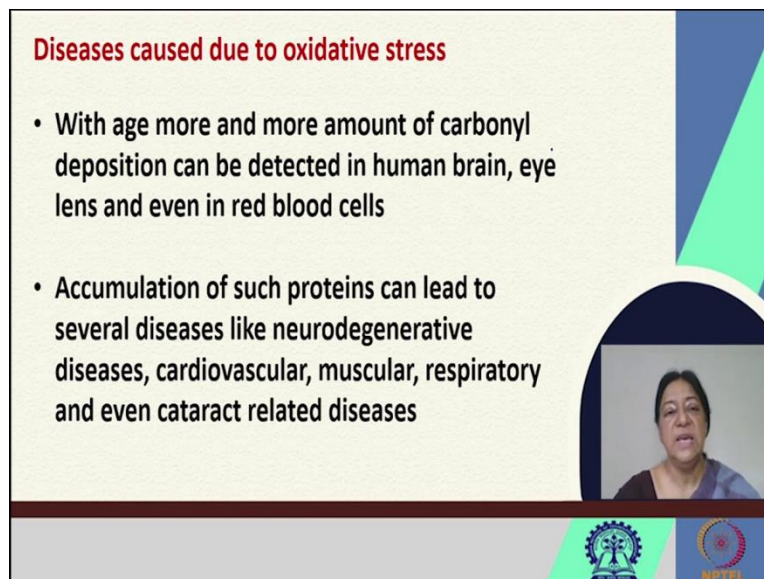
**Diseases caused due to oxidative stress**

- Exposure of proteins to ROS, heat, radiation etc can affect the enzymatic function and in various cases causes degradation of the protein structure
- Accumulation of various inactive or degraded proteins within the body with time is referred to as aging

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If we look at the diseases caused due to oxidative stress, there is an exposure of proteins to reactive oxygen species heat, radiation etcetera, that can affect the enzymatic function and in turn cause degradation of the protein structure. So the accumulation of various inactive or degraded proteins within the body with time, is referred to as an aging process.

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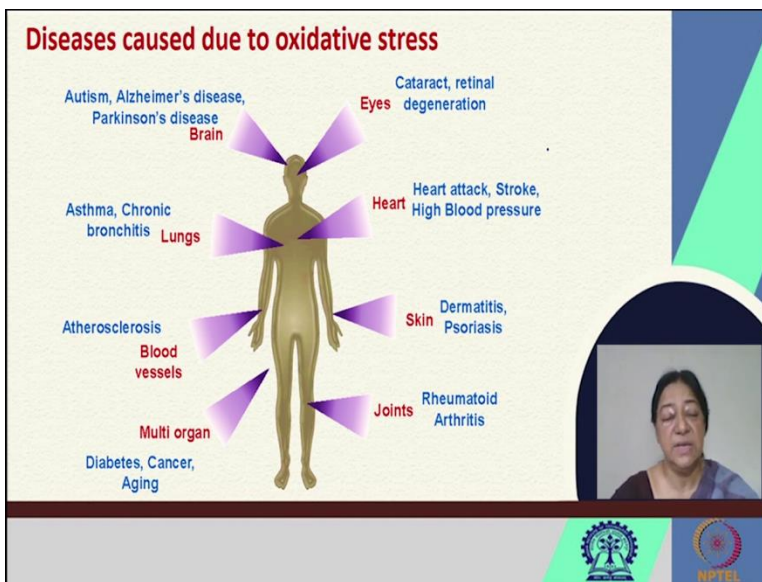
**Diseases caused due to oxidative stress**

- With age more and more amount of carbonyl deposition can be detected in human brain, eye lens and even in red blood cells
- Accumulation of such proteins can lead to several diseases like neurodegenerative diseases, cardiovascular, muscular, respiratory and even cataract related diseases

The slide features a light green background with a dark blue and light green geometric design on the right side. A video inset of a woman is positioned in the bottom right corner. At the bottom, there are logos for a university and NPTEL.

With age more and more amount of the carbonyl deposition can be detected in the brain, the eye lens and even in red blood cells. This accumulation of such modified proteins due to oxidative stress, can lead to various diseases.

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There are several diseases that are caused due to oxidative stress starting from cataract, Alzheimer's disease, asthma, specific heart attacks, stroke, atherosclerosis, diabetes, rheumatoid arthritis and so on.

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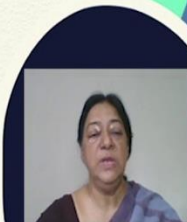
Diseases	Cause	Symptoms
Diabetes Mellitus	increase in blood glucose level	hunger, thirst, weakness
Parkinson's disease	modification of $\alpha$ -synuclein protein	unbalanced body movements, neuron damage

The specific proteins that are present and the causes due to the oxidative stress in proteins can lead to specific diseases and the specific symptoms are also given here [refer to slide].

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**Diseases caused due to oxidative stress**

Diseases	Cause	Symptoms
Alzheimer's Disease	deposition of the fibrillar aggregates of amyloid $\beta$ (A $\beta$ ) peptide	Memory loss
Multiple Sclerosis	ROS damages neurons	Central nervous system is affected



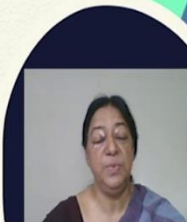


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**Diseases caused due to oxidative stress**

Diseases	Cause	Symptoms
Cardiovascular diseases	elevated levels of low density lipoprotein (LDL), free fat, glucose	harden the arteries-atherosclerosis
Cataract	Deposition of lens protein due to protein oxidation	Loss in lens transparency

Others include Rheumatoid Arthritis , Asthma, tumor formation etc



So we look at the deposition of specific amyloid peptide fragments, damaged neurons and also specific proteins that could be modified due to this oxidative stress process.



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**Biomarkers of oxidative stress**

N-Formyl Kynurenine (NFK) formation  
Dityrosine (DT) and Nitrotyrosine (NT) formation

How to prevent such oxidative damage?

Using small molecules/enzymes which will  
quench the free radicals or the oxidative species




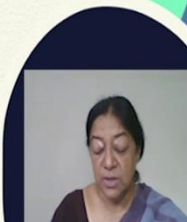
If we look at biomarkers of oxidative stress, we can look at NFK, dityrosine or nitrotyrosine formation. So the possibilities of modifications in proteins can occur through backbone modifications, can occur through specific amino acid modifications and these can lead to variations and disease.

The question is how to prevent such oxidative damage. This is possible by using small molecules of specific enzyme, that can quench these free radicals. But we also need to know that the presence of the free radicals is required for certain specific biochemical reactions.

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**Antioxidants** (like SOD, glutathione, Vitamin C, etc) can scavenge the excess free radicals and maintain a balance within the body

**Oxidative stress** is a condition when the **antioxidants** within our body is **not sufficient** enough to scavenge the free radical species generated from various sources



There are antioxidants like superoxide dismutase, glutathione, vitamin C etcetera, that can scavenge the excess free radicals and maintain a balance between the body. But oxidative stress

occurs when the antioxidants within the body are not sufficient enough to scavenge these free radicals that are generated by the various sources.

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**Oxidants and antioxidants**

Antioxidants are compounds that inhibit oxidation by scavenging the reactive species

Normal Conditions      During oxidative stress

NPTEL

So effectively what happens, in normal conditions there would be a balance between the antioxidant and the oxidant. However during oxidative stress, we have a predominance of the oxygens and the antioxidants are compounds that would inhibit the oxidation, by being able to scavenge the reactive species.

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**Antioxidants**

- Glutathione (GSH) found in plants, animals, fungi and some bacteria
- Ascorbic acid or vitamin C found in animals and plants
- Polyphenols: Compounds with multiple phenol units

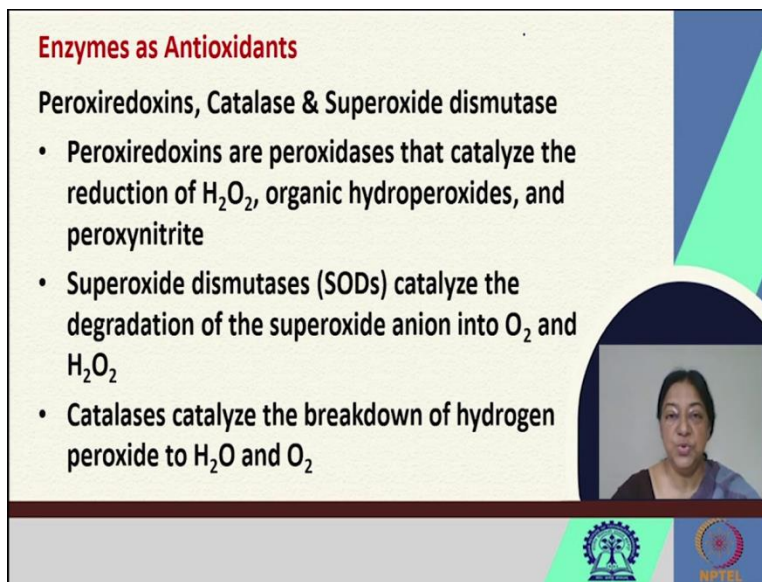
Epigallocatechin gallate (EGCG)

NPTEL

Glutathione as we just looked at, is found in plants, animal, fungi and some bacteria, is one such example. Ascorbic acid or vitamin C found in animals and plants and also polyphenols that are

compounds with multiple phenol units and some of these are a subject of a lot of research, where by looking at antioxidant activity on proteins we get an idea, either from dietary constituents or from enzymatic issues.

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**Enzymes as Antioxidants**

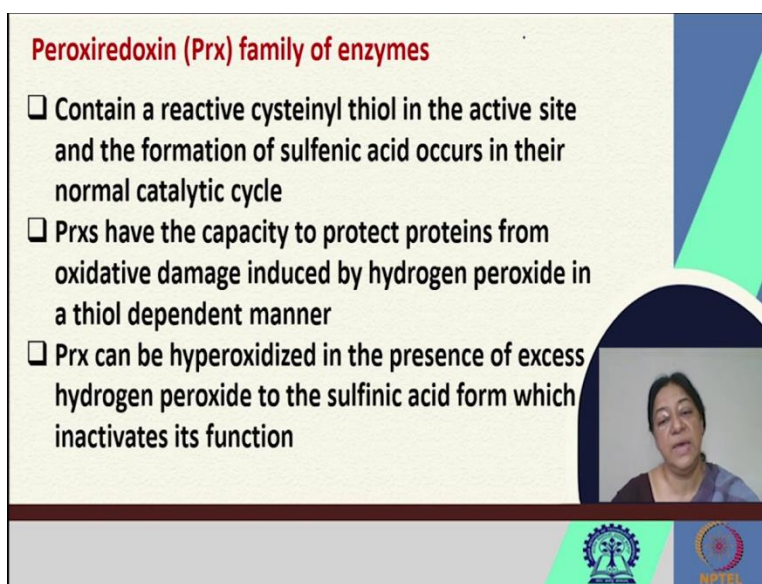
Peroxiredoxins, Catalase & Superoxide dismutase

- Peroxiredoxins are peroxidases that catalyze the reduction of  $H_2O_2$ , organic hydroperoxides, and peroxynitrite
- Superoxide dismutases (SODs) catalyze the degradation of the superoxide anion into  $O_2$  and  $H_2O_2$
- Catalases catalyze the breakdown of hydrogen peroxide to  $H_2O$  and  $O_2$

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

If we look at enzymes as antioxidants, there are peroxiredoxins, catalase and superoxide dismutase. Peroxiredoxins are peroxidases that catalyze the reduction of  $H_2O_2$  organic hydroperoxides and peroxynitrite. Superoxide dismutases, these catalyze the degradation of the superoxide ion into oxygen and  $H_2O_2$ . The catalases catalyze the breakdown of hydrogen peroxide to  $H_2O$  and  $O_2$ .

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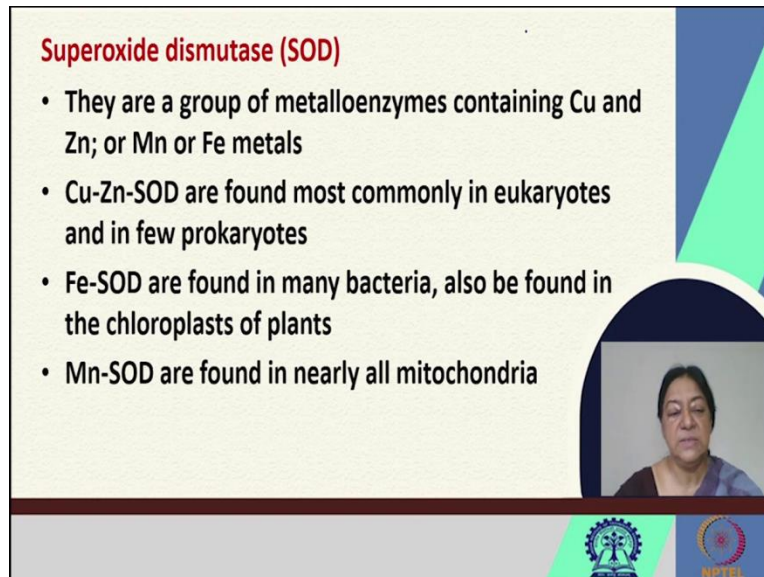
**Peroxiredoxin (Prx) family of enzymes**

- Contain a reactive cysteinyl thiol in the active site and the formation of sulfenic acid occurs in their normal catalytic cycle
- Prxs have the capacity to protect proteins from oxidative damage induced by hydrogen peroxide in a thiol dependent manner
- Prx can be hyperoxidized in the presence of excess hydrogen peroxide to the sulfinic acid form which inactivates its function

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

So if we look at the Prx family of enzymes, these contain a reactive cysteinyl thiol in the active site and the formation of sulfenic acid occurs in their normal catalytic cycle. These have the capacity to protect proteins from oxidative damage induced by hydrogen peroxide in a thiol dependent manner and they can be hyperoxidized in the presence of excess  $H_2O_2$  to the sulfinic acid, which again inactivates its function.

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**Superoxide dismutase (SOD)**

- They are a group of metalloenzymes containing Cu and Zn; or Mn or Fe metals
- Cu-Zn-SOD are found most commonly in eukaryotes and in few prokaryotes
- Fe-SOD are found in many bacteria, also be found in the chloroplasts of plants
- Mn-SOD are found in nearly all mitochondria

The slide features a light green background with a dark blue and light green geometric design on the right side. A small video inset in the bottom right corner shows a woman speaking. At the bottom of the slide, there are two logos: a circular emblem on the left and a logo with the letters 'WPI' on the right.

So there is a very delicate balance. If we look at superoxide dismutase, these are the group of metalloenzymes that contain copper, zinc or manganese and iron. Copper zinc superoxide dismutase are found most commonly in eukaryotes and in few prokaryotes and we have the variations in terms of the metals that are there in the superoxide dismutase. Some can be found in bacteria in plants and some in mitochondria.

(Refer Slide Time: 23:24)

**Superoxide dismutase (SOD)**

- They catalyze the disproportionation of superoxide:
 
$$2 \text{HO}_2 \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$$
- They are the only antioxidant enzyme that scavenges the superoxide anion through the disproportionation reaction

PDB ID: 1SDY

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This [refer to slide] specific protein catalyzes the disproportionation of superoxide into  $\text{O}_2$  and  $\text{H}_2\text{O}$ . They are the only antioxidant enzymes that are capable of scavenging the superoxide anion through this disproportionation reaction.

(Refer Slide Time: 23:45)

**Superoxide dismutase (SOD)**

- Zn coordinated by 3 His and 1 Asp
- Cu coordinated by 4 His and 1  $\text{H}_2\text{O}$
- Cu(II) and Zn(II) is bridged by imidazolate ring of His 63

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So, if we look at the specific active side as we saw in metalloproteins, we have the coordinations through the specific histidine residues and a specific activity due to this coordination. It is coordinated by three histidines and one aspartic acid, the copper is coordinated by 4 histidine and 1  $\text{H}_2\text{O}$ . So, this is a copper zinc SOD superoxide dismutase and the Cu(II) and the Zn(II) are bridged by the imidazolate ring of His 63, as shown here [refer to slide].

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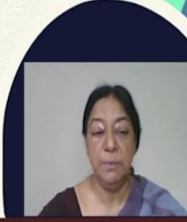


**Oxidative stress on monoclonal antibodies (mAbs)**  
Various degradation pathways that can impact the higher order structure (HOS) of monoclonal antibodies (mAbs)

Chemical modifications: Oxidation

Oxidation of amino acids:

- Methionine, tryptophan, etc oxidation caused either by direct light exposure or by secondary impacts of free radicals



The oxidative stress on monoclonal antibodies is another important factor. There are various degradation pathways as we saw, that can impact the higher order structure of monoclonal antibodies. We had seen the structure of antibodies previously and how they are important in antibody antigen interactions.



So, chemical modifications in terms of oxidation may be possible and we looked at the oxidation of specific amino acids. Here for example methionine, tryptophan etcetera oxidation that can be caused by direct light exposure or by secondary impacts of free radicals.

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**Oxidative stress on monoclonal antibodies (mAbs)**

Deamidation:

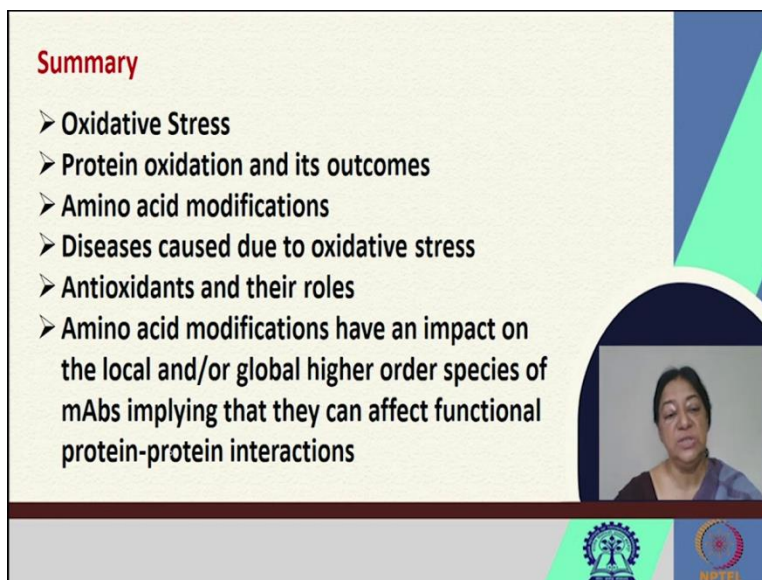
- It is a spontaneous reaction that leads to the conversion of asparagine to aspartate/isoaspartate/succinimide intermediate
- It depends on the pH and solution conditions
- Primary sequence and higher order structure can also cause deamidation intrinsically



In case of deamidation, this is a spontaneous reaction that leads to the conversion of asparagine to aspartate, isoaspartate, succinimide intermediates. These depend upon the pH as well as the

solution conditions and the primary sequence and higher order structures can also cause intrinsic deamidation, which is an important factor in oxidative stress of these antibodies.

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**Summary**

- Oxidative Stress
- Protein oxidation and its outcomes
- Amino acid modifications
- Diseases caused due to oxidative stress
- Antioxidants and their roles
- Amino acid modifications have an impact on the local and/or global higher order species of mAbs implying that they can affect functional protein-protein interactions

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In summary what we looked at, we looked at oxidative stress in proteins, we understood that there were specific possibilities for the damage due to oxidative stress in terms of backbone modification, specific amino acid modifications that could also be used as biomarkers to understand the extent of oxidative stress.

The protein oxidation and its specific outcomes and how we have specific diseases due to these amino acid modifications and the role of antioxidants in combating any oxidative stress that may occur. The amino acid modifications we learnt have an impact on the local and/or global higher order species of the monoclonal antibodies and this is important because this means that they can affect functional protein-protein interactions.

In this specific topic or the specific topics that we will be considering for this module, are more research oriented to give us an idea of the open problems in protein chemistry. Not all of them are possible to be discussed here, but nevertheless to get some idea, the specific references and specific books available will be given.

Thank you.