Bioinorganic Chemistry
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Lecture - 07
Design Principles Used in Chemical Biology: Role of Protein Chains in Controlling Reactivity

Hi everybody and welcome back to our discussion on Design Principle Used in Chemical Biology. We have been discussing how the beautiful design principle adopted by our mother nature, control many key processes in our life. In my previous lecture, we have discussed the dioxygen carrying Proteins in Biology.

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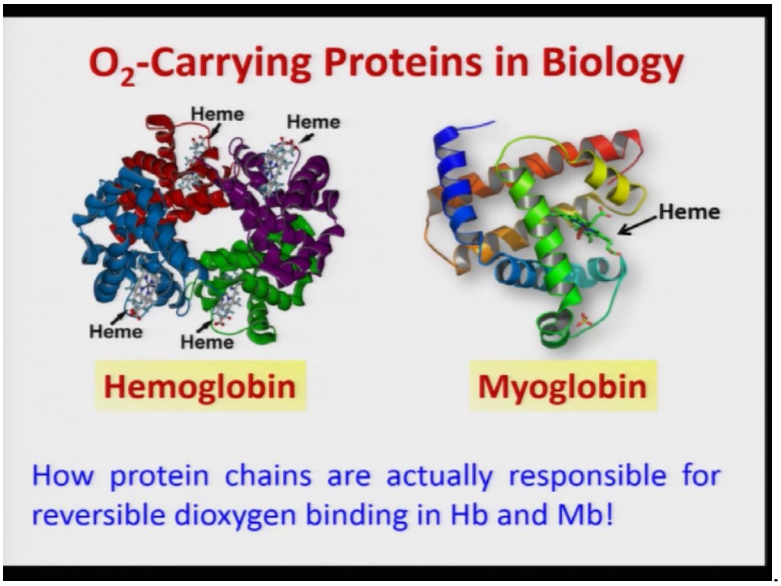

I have illustrated how protein chain actually responsible for reversible dioxygen binding in hemoglobin and myoglobin in our body.

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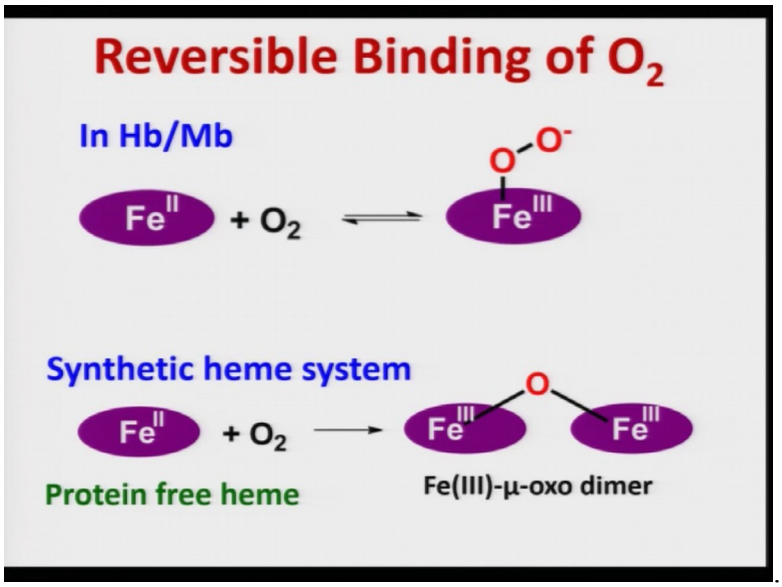

As you can see that hemoglobin and myoglobin carries dioxygen reversibly, Fe(II) is oxidized toFe(III); where dioxygen is reduced by one electron to superoxide O_2^- and this binding is reversible in our body.

In contrast, if one have the synthetic heme system without protein chain, then also this dioxygen converts this Fe(II) heme center to a mu oxo dimer which is no longer able to bind dioxygen. It is a very stable complex and thus in synthetic heme centers, the dioxygen it cannot be carried reversibly.

This I have discussed in details in my previous lecture.

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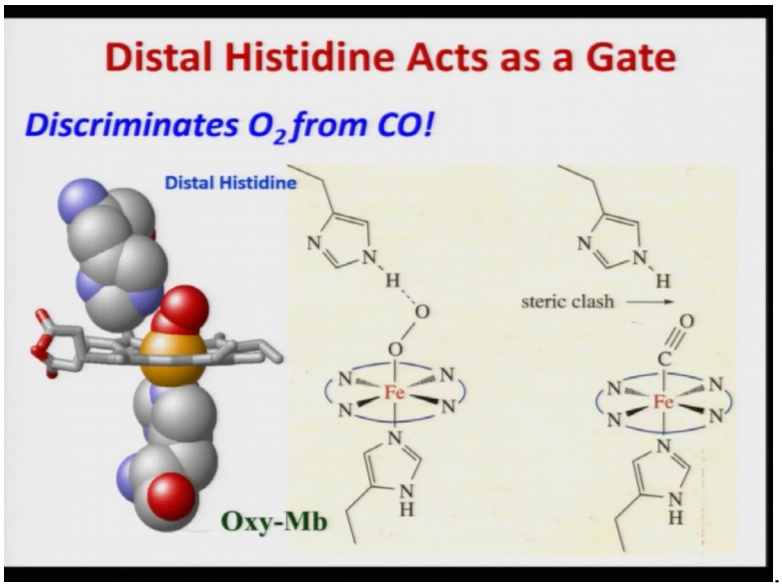

I also have discussed that the importance of design in discriminating oxygen from carbon monoxide just by using Distal Histidine as a gatekeeper. As you can see that, while dioxygen comfortably fits inside the cavity, indeed it also facilitates by the hydrogen bonding with the distal histidine. In such contrast, carbon monoxides are forced to bind in an angular fashion and thereby this binding becomes very weak.

And that is why patients having carbon monoxide poisoning can be treated very easily by just taking that patient to fresh air.

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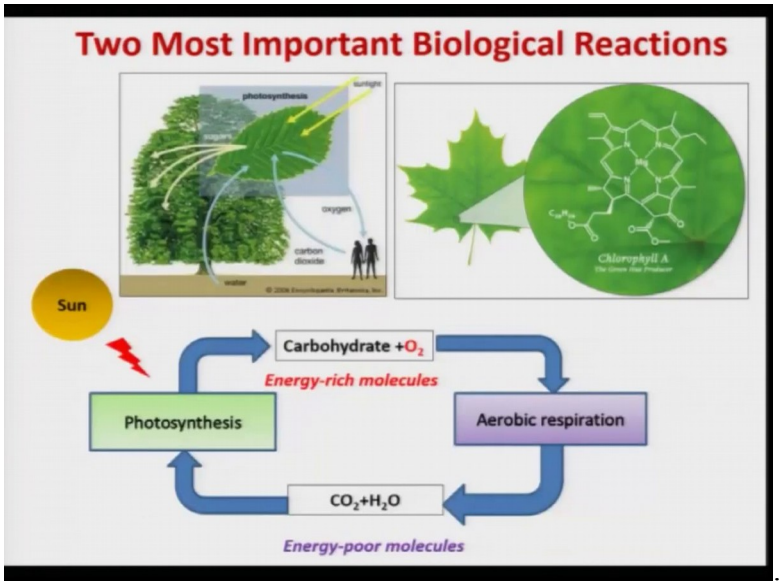

Two most important biological reactions; one is as you know that photosynthesis and respiration.

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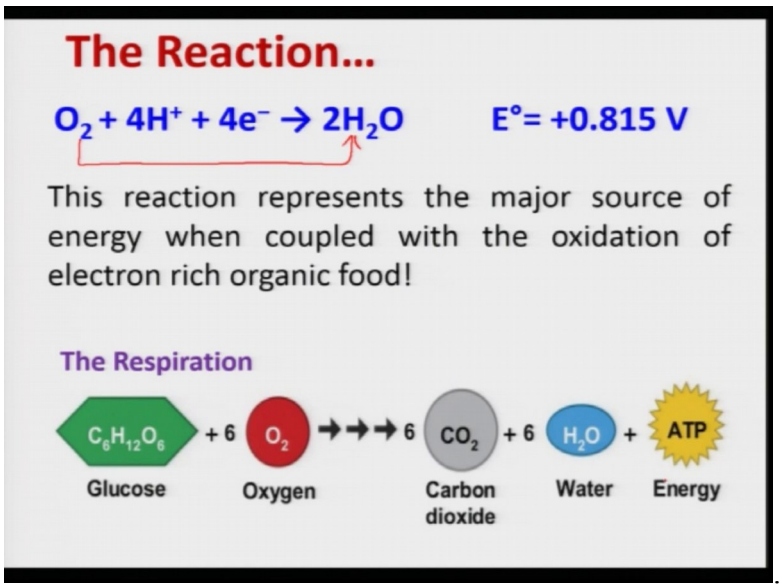

This respiration releases huge amount of energy out of this process and the main reactions which is responsible for this huge amount of energy is the respiration and what is happening in the respiration is, the dioxygen is converted to water with the help of 4 proton and 4 electron.

The E^0 values is +0.815 volt. This reaction represents the major source of energy when coupled with oxidation of electron rich organic food. The main reaction is shown here, this glucose plus 6 molecule of oxygen eventually converts to 6 molecule of carbon dioxide, 6 molecule of water and releases huge amount of energy which are stored in ATP.

Now, as I have just said this dioxygen converting to water required 4 proton and 4 electron in a concerted manner; means all at a time to produce this water.

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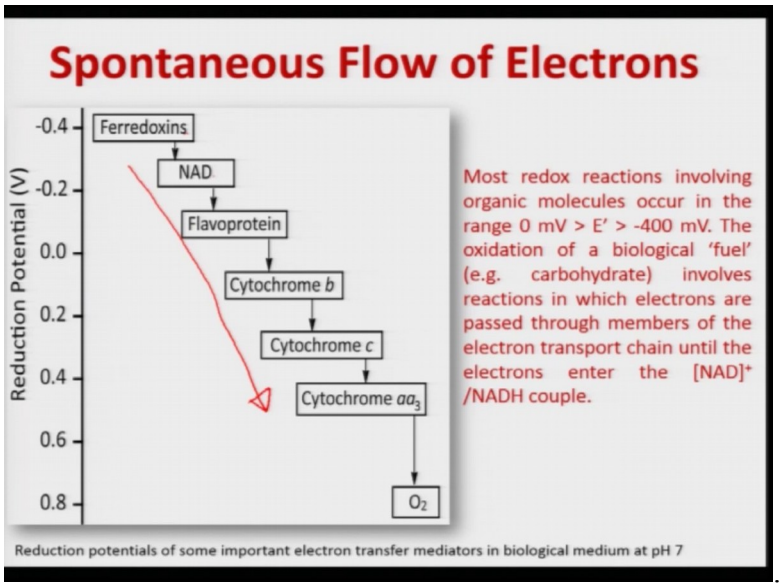

Now, this large number of electron comes spontaneously like this, say most redox reactions involving organic molecules occur in the range of 0 to -400 millivolt. And this electron comes to ferredoxin, then ferredoxin transfer electron to NAD and then ferredoxin become oxidized.

Now, once NAD gets the electron, NAD becomes in reduced state and immediately gives electron to flavoprotein, while NAD becomes oxidized. So, this flow is spontaneous like this and eventually it comes to cytochrome c. Cytochrome c gives electron to cytochrome aa_3 and cytochrome aa_3 transfer electron to dioxygen. It is the terminal enzyme, where actually this dioxygen reduction, this 4 electron reduction is happening and it releases huge amount of energy out of that.

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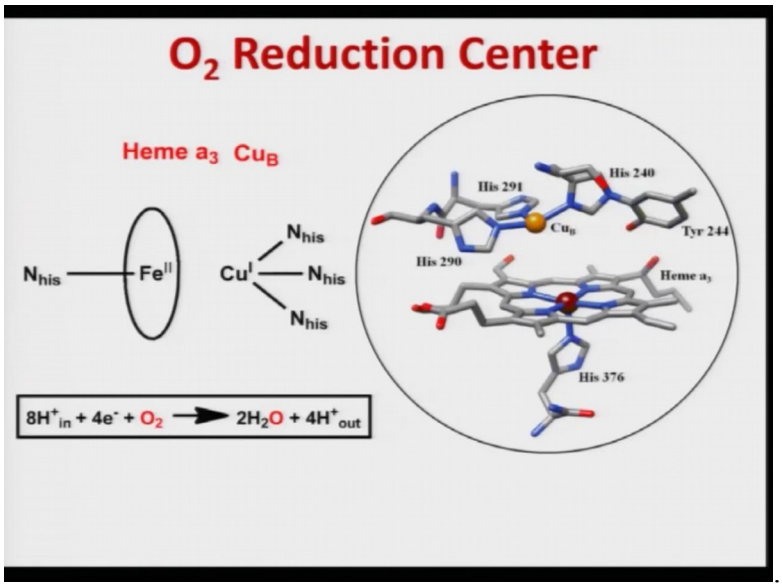

So, the center where dioxygen indeed getting reduced to water, is that cytochrome c oxidase and indeed this is heme a_3 and $copper_B$ is there where dioxygen comes and binds. I will be discussing these all in details after a week in the subsequent lectures; however, this releases huge amount of energy.

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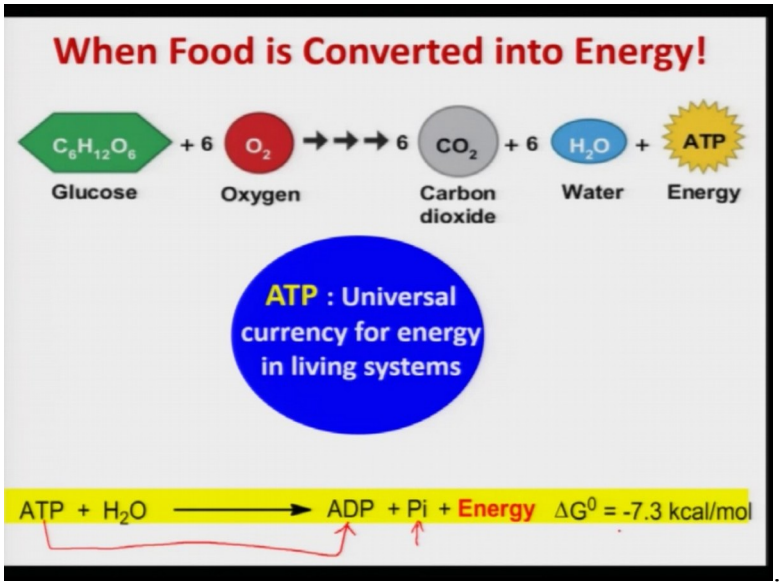

What would happen when food is converted into energy, when you have huge amount of energy released out of the respiration as I have just said; that ATP stores energy.

So, ATP Adenosine Triphosphate is an universal currency for energy in living systems. Now whenever the system need energy, it just hydrolyze ATP, converts to ADP Adenosine Diphosphate plus inorganic phosphate; and then releases huge amount of energy which we actually utilize for our day to day activity.

So, ATP actually is responsible for storing all sorts of energy which is produced out of respiration; and then when there is a need for energy, it undergoing hydrolysis.

Kinase is the enzyme which is responsible for hydrolysis and it releases energy out of this reaction as shown over here.

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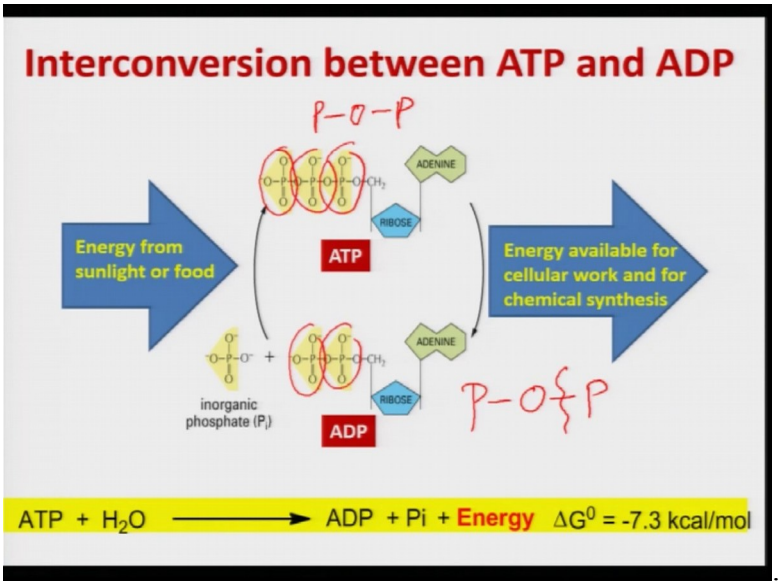

Now, as I have said that when ATP stores the energy and releases the energy when it is required. So, I will be now showing the inter conversion between ATP and ADP. As one can see this ATP adenosine triphosphate there are the 3 phosphate group which are linked P -O -P; you see that this is one phosphate, this is another phosphate, and this is another phosphate, three phosphates and linked by P -O -P bond. When there is a need for energy, it gives the energy and converts to ADP adenosine diphosphate; as you can see that there are two phosphate group, one and two and releases another phosphate.

So, what happens, P -O -P bond breaks or hydrolyzed and then this energy comes out of this, bond breaking process is utilized for our day to day work for our life. The energy

comes from either sunlight or food when we get energy what happens, again this P –O- P bond forms.

So, it thereby stores as ATP. So, you can see that ATP and ADP undergo a cyclic interconversion and when energy comes, it stores in the form of P -O -P bond and it releases energy out of breaking P -O -P bond. Indeed this is what is very important, and this is what is happening in our day to day life all the time.

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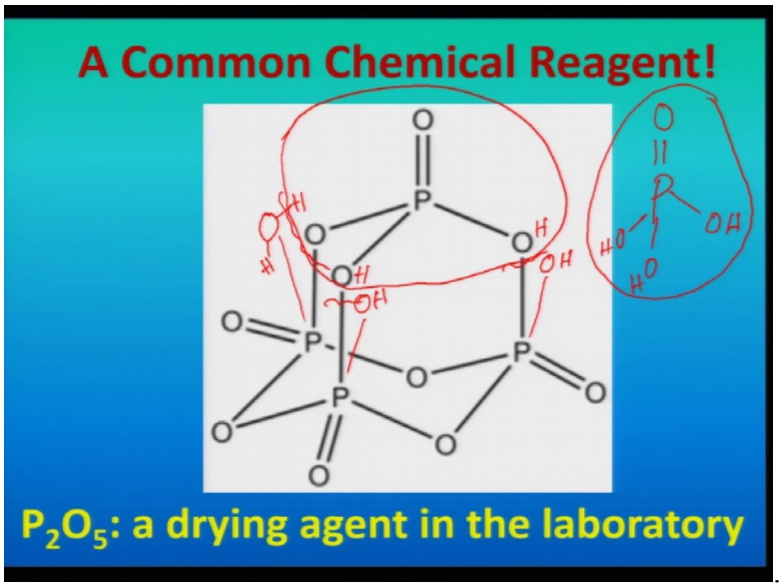

You can see phosphorus pentoxide is a common drying agent in the laboratory, almost every laboratory have this phosphorus pentoxide. And the phosphorus pentoxide structure is shown over here; you can see there, there are several P -O -P bond, which indeed can be hydrolyzed, gives rise to lots of energy ,that is indeed happening and I will now demonstrate how this molecule releases huge amount of energy out of such P -O -P bond hydrolysis, as also observed while ATP converts to ADP in biology ok.

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So, I will now show how the or hydrolysis of P -O -P bond produces huge amount of energy. You see this is phosphorus pentoxide, and it is a white powder and I am opening that bottle is air tight and this is simple water; I will use this spatula and put some of this phosphorus pentoxide and I will keep it in this watch glass. Please note that this phosphorus pentoxide is hydroscopic and you will see soon, what would happen. So, this little bit phosphorus pentoxide I have added here, and just addition of little bit of water will produce huge amount of energy.

You can see yourself. You can see that just additional small amount of water produces how much energy, this plate is very hot; it displays that lots of energy is being released. Now what is exactly happening, that when you add a drop of water into this phosphorus pentoxides. This water actually hydrolyzed this like H and OH and this P O bond get it breaks and it forms OH. Similarly this also forms OH out of such break; and of course O H is there, OH is there which actually forms bond to this phosphate.

And so, what is exactly happening, you see that this is what is happening; you have a phosphoric acid this is what is happening OH OH and OH, this phosphoric acid is being formed at the end. Now you see that there are so many P- O -P bonds which can break stepwise and releases huge amount of energy and that I have just shown you. You can indeed correlate what is happening in biology and try to understand the basic chemistry out of such hydrolysis process ok.

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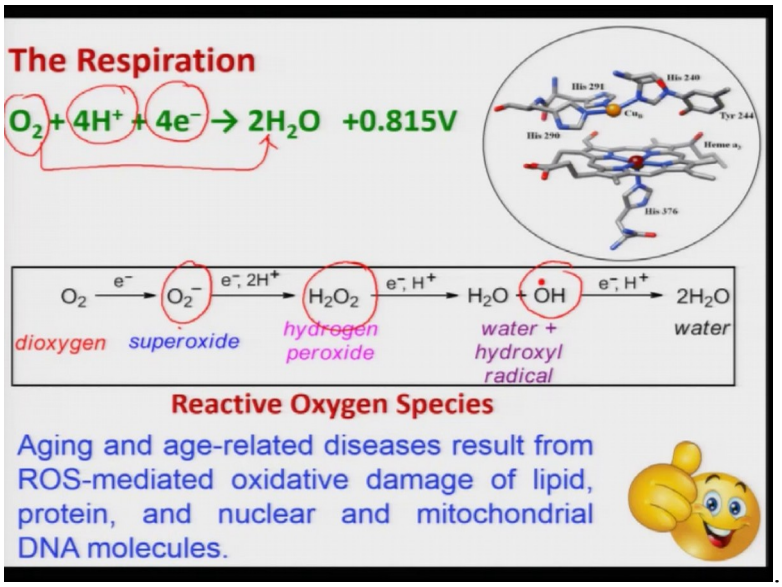

So, coming back to this respiration again, what is happening as I have just said that oxygen, aerial oxygen is converted to water with the help of 4 protons and 4 electrons; and this reaction actually helps to get huge amount of energy in the respiration, which we have been utilizing for our day to day activity. Now the beauty of this reaction is that the, this conversion of oxygen to water, if it have to be in one step; like 4 protons and 4 electrons have to be added all at a time to reduce dioxygen completely to water.

Now, if there is an incomplete reduction, indeed that also happens, nature also do mistake and incomplete reduction like then dioxygen after one electron reduction from superoxide O_2^- and superoxide after one electron reduction forming O_2^{2-} peroxides and peroxides after one electron reduction it is also gives rise to water and hydroxyl radical and eventually another reduction converts to 2 molecule of water. Indeed this superoxide, peroxides and hydroxyl radical all collectively is called Reactive Oxygen Species ROS, , which is actually poisonous for our body.

And lots of problems comes out of that, one such problem is; aging and age related disease, which actually occurs out of this ROS mediated oxidative damage of lipids, protein, and nuclear and mitochondrial DNA molecule.

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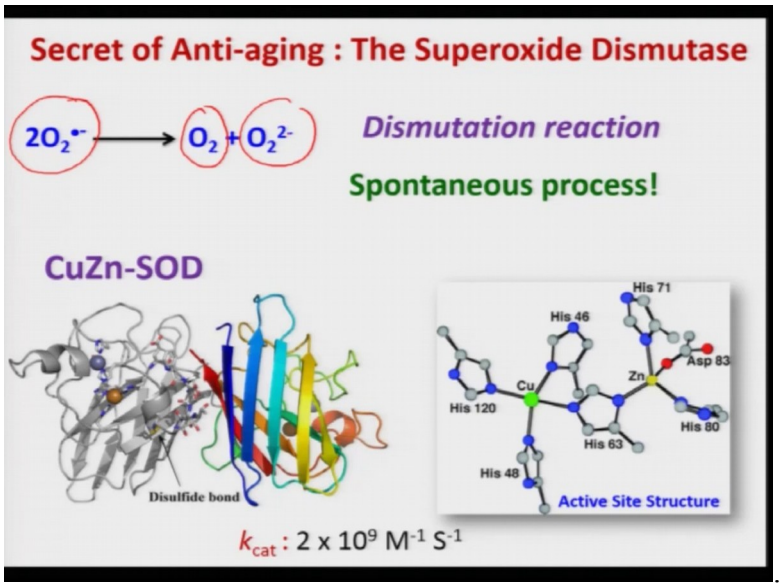

The secret of such an Anti-aging is the superoxide dismutase. So, nature, as I have said the incomplete reduction of dioxygen also happens in the biological system; but nature's design is such that, even if undesired product nature utilizes, nature has a solution for it; and this is what is the solution in superoxide dismutase.

What is happening, the superoxides converts to dioxygen and peroxide and this is a dismutation reaction and it is a spontaneous process. ΔG is large negative. So, it is a spontaneous process and still nature designed an enzyme called superoxide dismutase, just to speed up rate of this reaction and as you can see that when this enzyme is used rate of reaction becomes$2x10^9$ M^{-1} S^{-1}.

So, it is increased by many 1000 volts ok. Please also note that superoxide dismutase also produce peroxides, which is another biological poison and nature has a solution for it.

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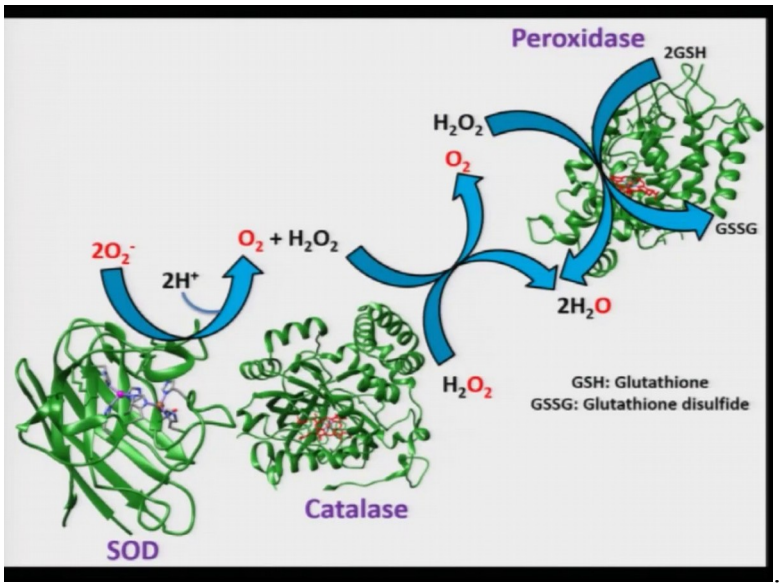

Nature thereby design other enzymes like catalase and peroxidase, which takes care of this peroxides and convert to something very useful, oxygen and other products which are useful for our day to day life.

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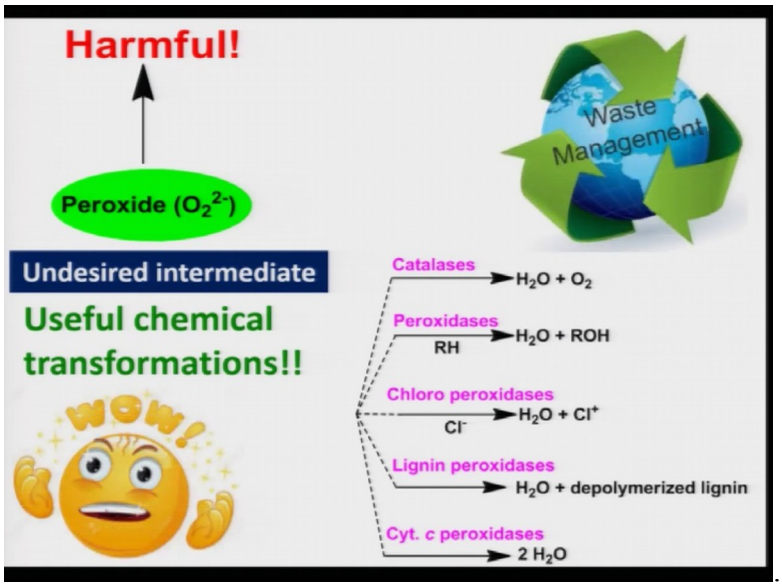

Indeed this peroxide, which is indeed undesired intermediates and also very harmful for our body ; converts to something very useful product by several enzymes like catalase, peroxidase, cytochrome c peroxidase, lignin peroxidase, chloro peroxidase, all these

products which are being formed out of those catalases are very very crucial for our life and now you see that, what could be a better waste management than this.

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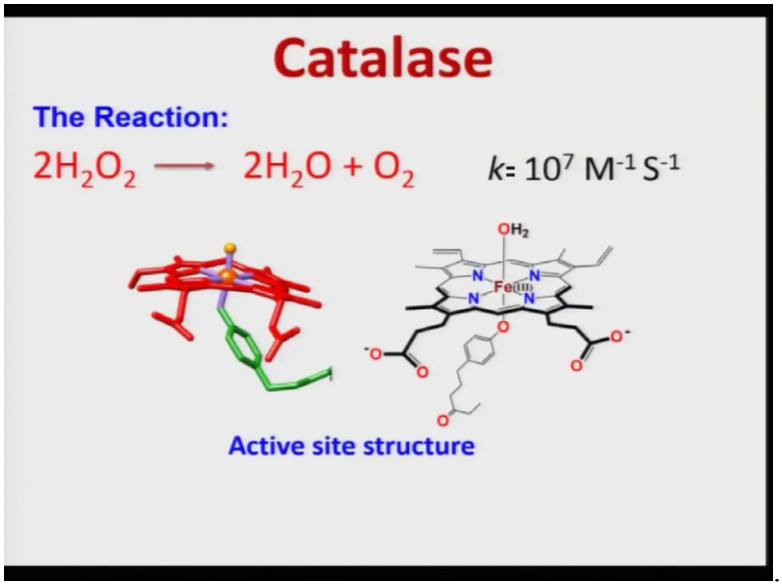

Let us look at how catalase actually works, as I said that catalase converts this hydrogen peroxides to water and oxygen.

Peroxides are extremely poisonous, it converts to all bio friendly water and dioxygen and k catalase is 10^7 M^{-1} $S^{-1}$and the active site structures are shown over here, I will be discussing these in details in subsequent lectures.

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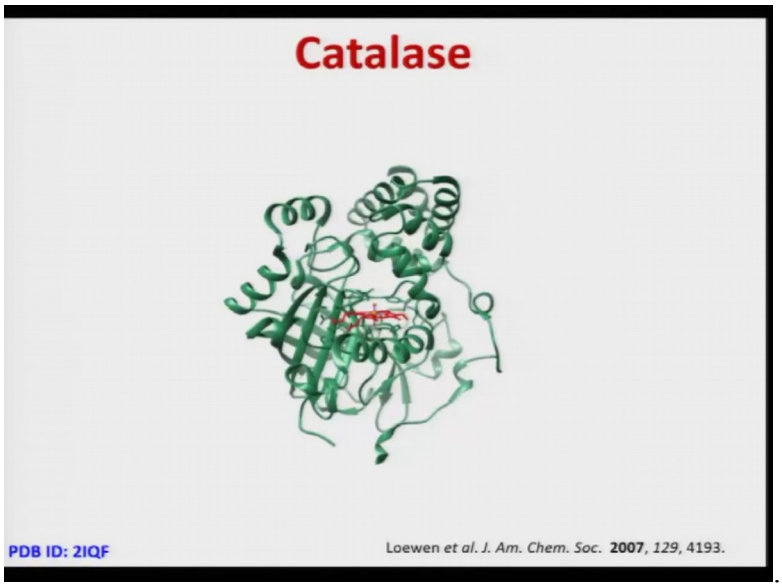

So, X-ray structure of catalase is shown over here, as one can see that this heme center is surrounded by huge protein chains and you can see that, there is a tyrosine at the fifth position, water molecule is at the sixth position and heme centers also clearly visible and this iron is sitting at the center of this porphyrin macro cycle.

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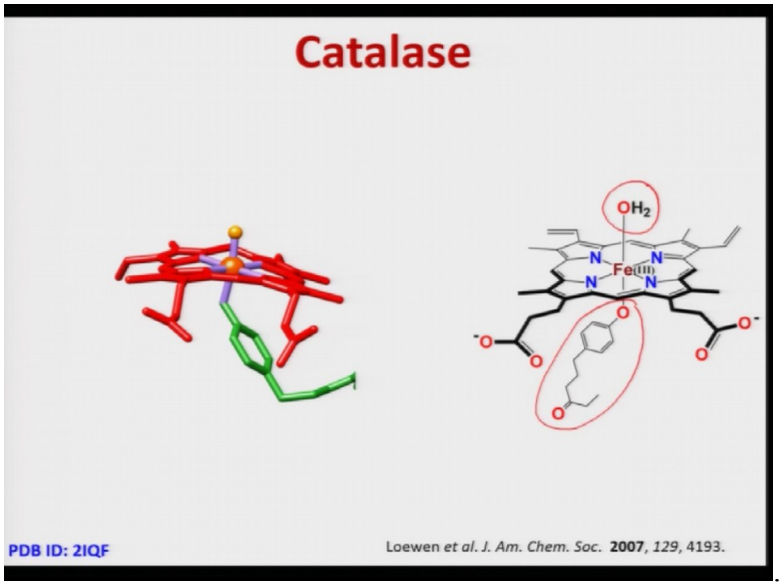

Now when you remove this protein, you can see this active site structure very clearly; we will discuss in more details in subsequent lecture.

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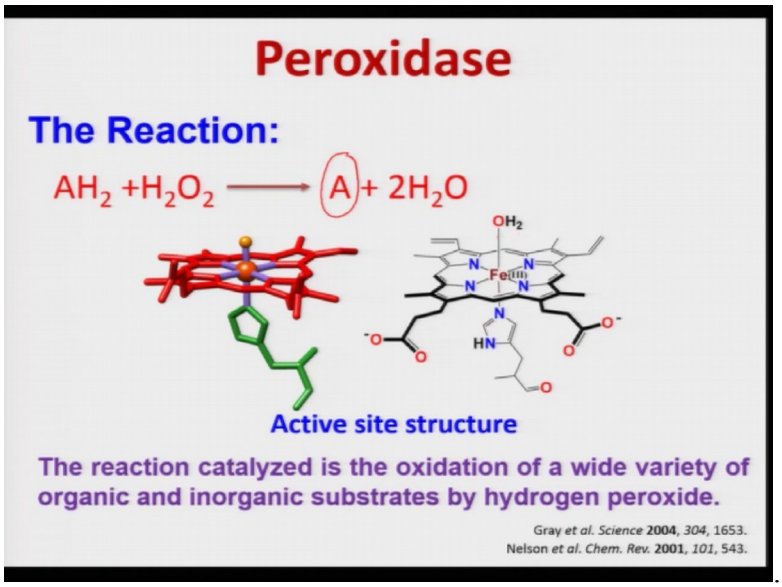

Now, peroxidase is another enzyme which actually catalyze the oxidation of a wide variety of organic and inorganic substrate by utilizing this hydrogen peroxides. Please note that, these peroxides is undesired intermediate.

So, peroxidase, utilize those undesired intermediate to something very useful products which is otherwise impossible to make in the biological system. So, the active site structures are shown over here and as you can see that this iron is in the middle, this is also Fe(III) and this is the heme center and the fifth position is occupied by a imidazole group and so, it is an imidazolate form and 6^{th} position again the weak water molecule, which is ligated to the Fe(III) center.

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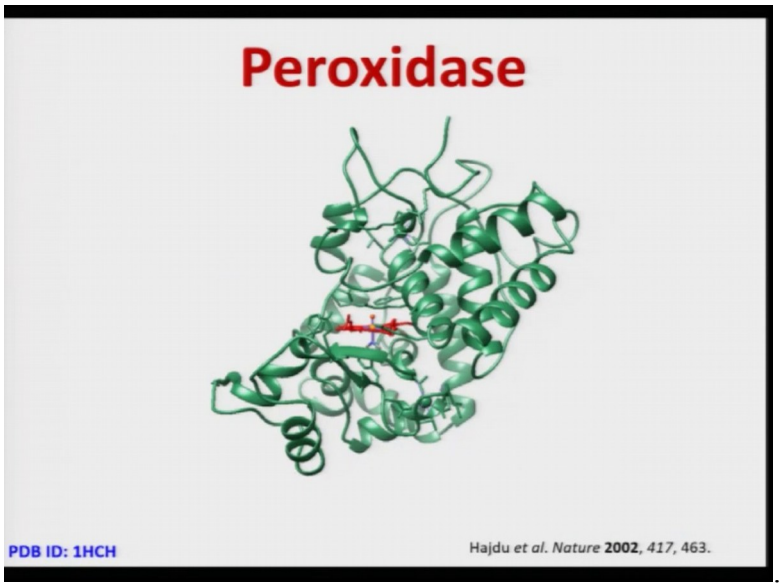

And X-ray structure of peroxidase is shown over here; as one can see very clearly that huge protein chains are wrapping this heme centers and they actually dictate the substrates, what kind of substrate would come and the specificity of the reaction; I will be showing you very soon in details.

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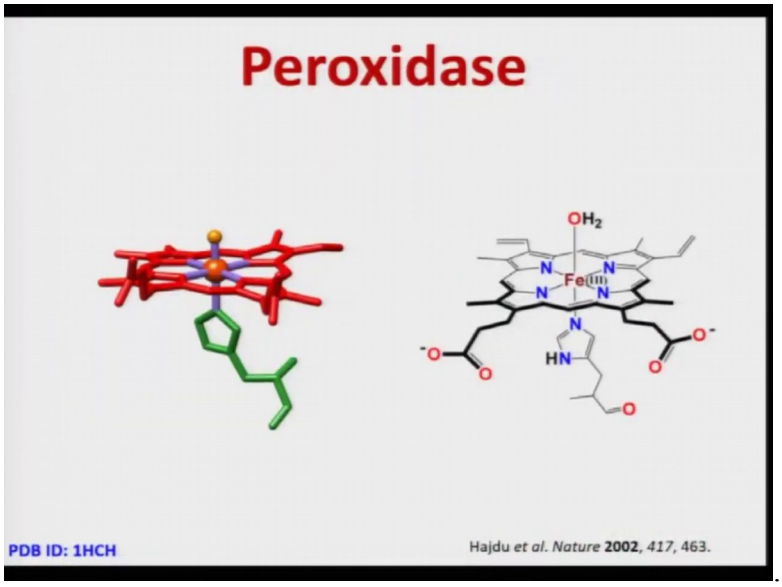

Once you remove these protein chains, it gives this active site structure as one can see this; this is iron 3 heme centers.

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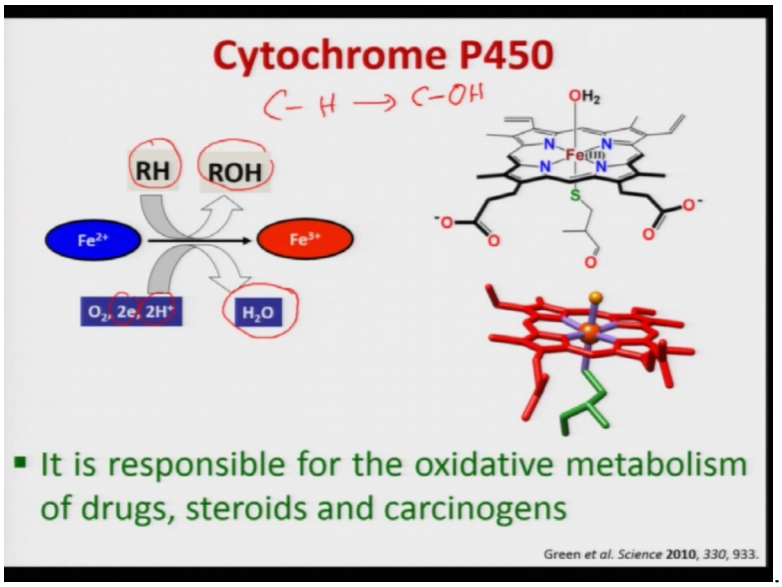

Cytochrome P450, another extremely important enzyme for our day to day life. Indeed it is responsible for the oxidative metabolism of drugs, steroids and carcinogens and thereby helping our life.

And although I will be talking in details in my subsequent lectures, but I like to focus here, the design principles; as one can see that during this transformation what is happening, this RH converted to ROH with the help of just aerial oxygen and 2 electrons and 2 protons and which yield also the water, which is bio friendly.

So, these kind of reaction, the large number of reactions are happening in our biological system, in our body every time and cytochrome P450 is responsible for this types of conversion. You see oxo group is getting inserted between C-H bond. So, C-H bond converted to C-OH bond, we will be looking this kind of reaction in details in subsequent lectures.

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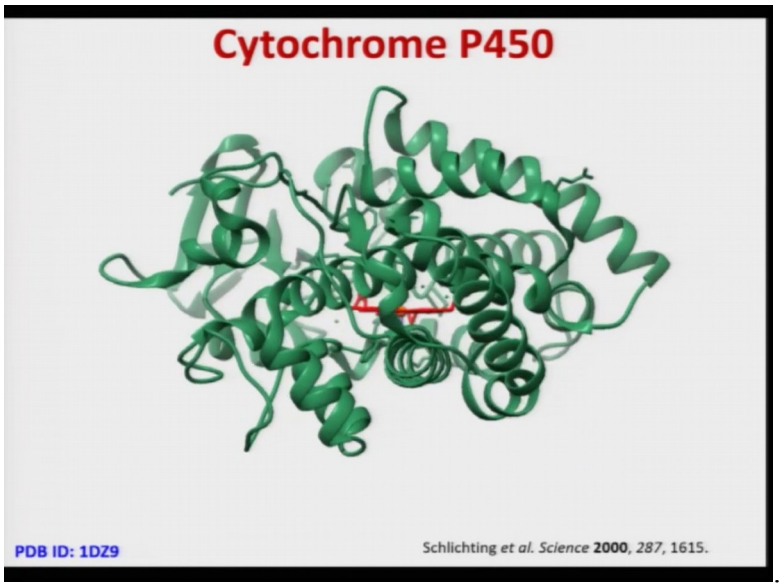

Now, the X-ray structure of cytochrome P450 is shown over here, once again as you have seen in case of catalase and peroxidase, a huge protein chainsurrounding the heme centers; of course I am going to highlight the importance of this large protein chains.

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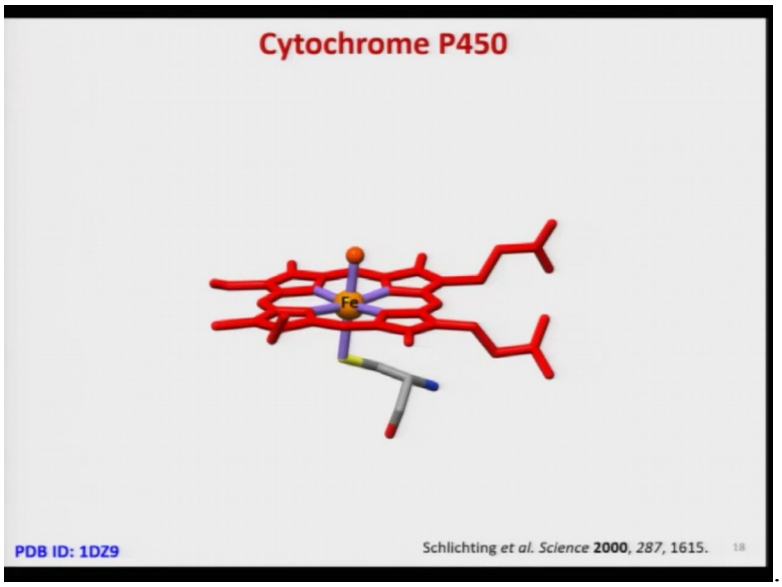

You will see soon, once the protein chains are removed, you can see the heme center which is actually the active site structure of cytochrome P450. The fifth position is bound with cysteine sulfur, and sixth position is a weak water molecule coordinated to the iron

center and iron isFe(III), and just like very similar as you have seen in case of catalase, peroxidase also.

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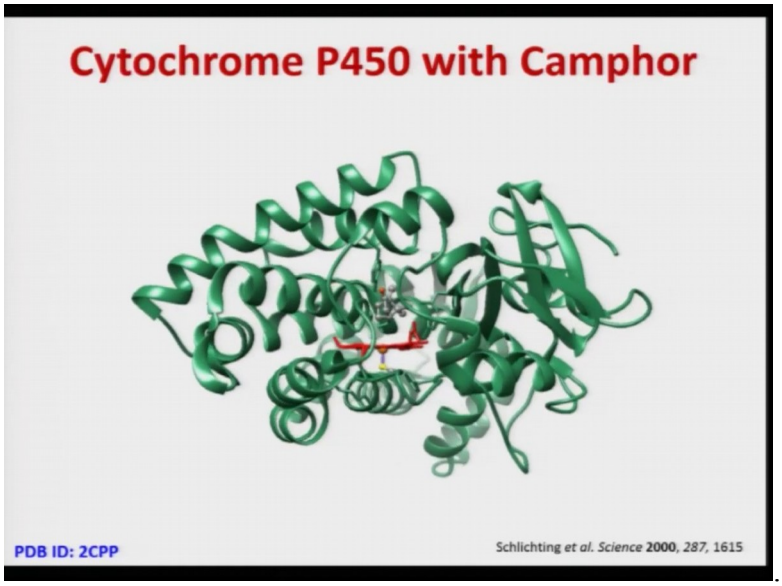

Here is the X-ray structure of cytochrome P450 with Camphor as a substrate. You can see where this camphor is sitting, is very interesting; you know this has an implications,I will be showing shortly to that. You see that this camphor is just sitting top of this iron centre and the closest carbon is getting hydroxylated.

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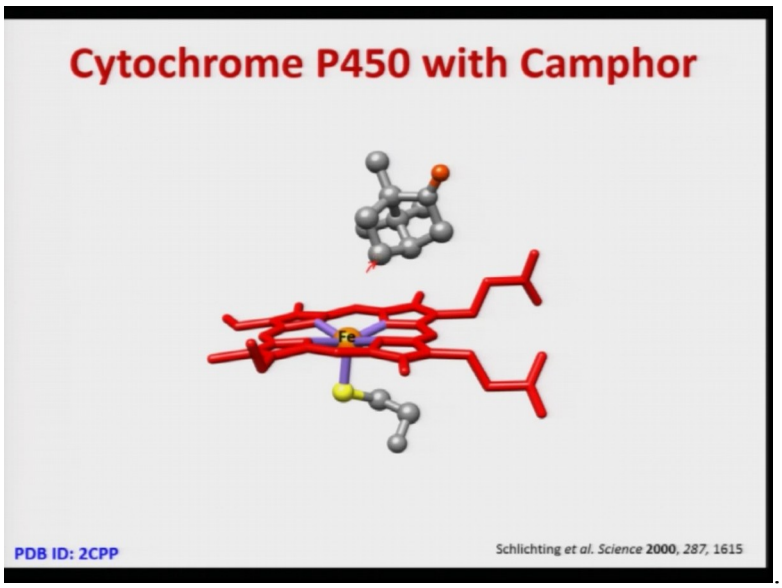

So, once you remove this protein chains, it would be clear. So, camphor is sitting and the closest carbon is this, which are getting hydroxylated.

Because iron, I will be showing shortly; this Fe(IV) oxo is getting formed which is very reactive and undergo a hydroxylation process and this is what happens, it forms 5-Exo-hydroxycamphor.

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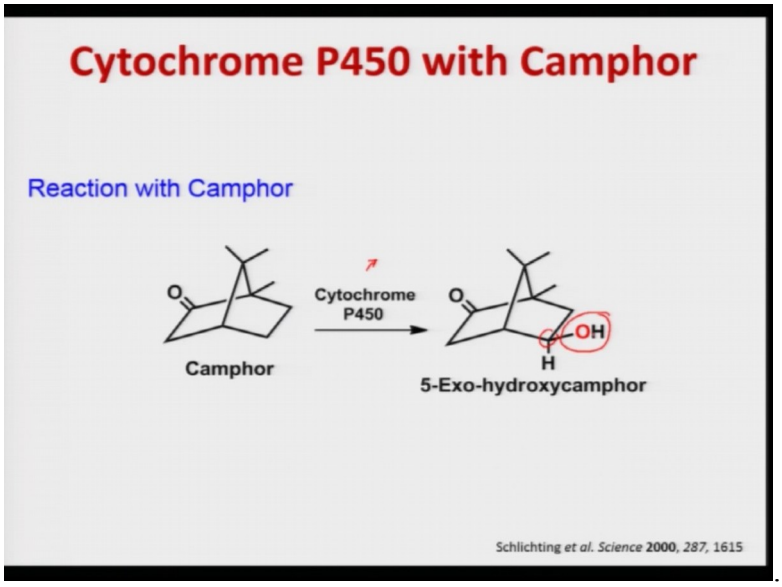

And this is the closest carbon to the iron and it is also in the Exo not in the Endo.

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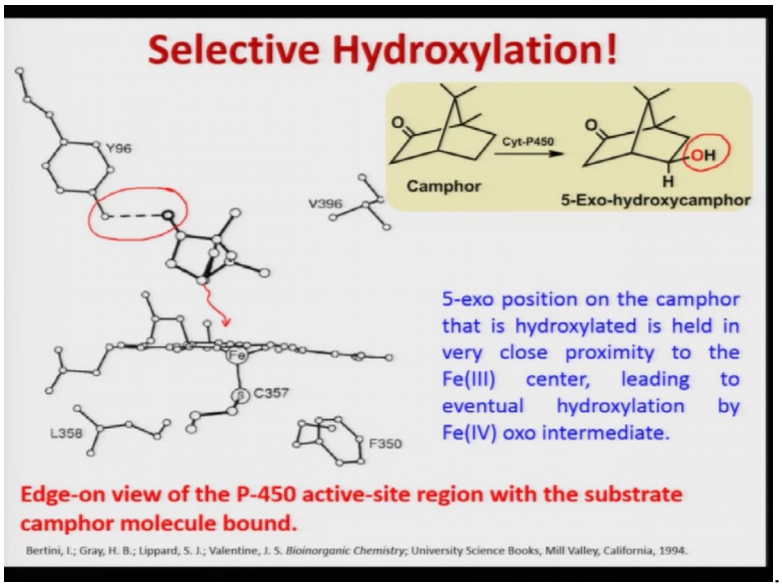

Now, let us look at that, why such a hydroxylation process is so selective and this is the x-ray structure of cytochrome P450 with camphor as a substrate and as you can see that substrate camphor is located in a hydrophobic pocket, directly above the heme.

And it is oriented by a hydrogen bond between carbonyl oxygen of the camphor and tyrosine 96. You see that this is what is the hydrogen bonding, which actually direct this camphor; the position that is hydroxylated in the oxygenation reaction is 5-exo position, the closest point of approach of the substrate to the expected position of the oxygen atom, bound to iron in the high valent iron oxo intermediate.

So, this 5-exo position is the closest to this iron center and this is highly stereospecific because of this hydrogen bonding as I have just discussed.

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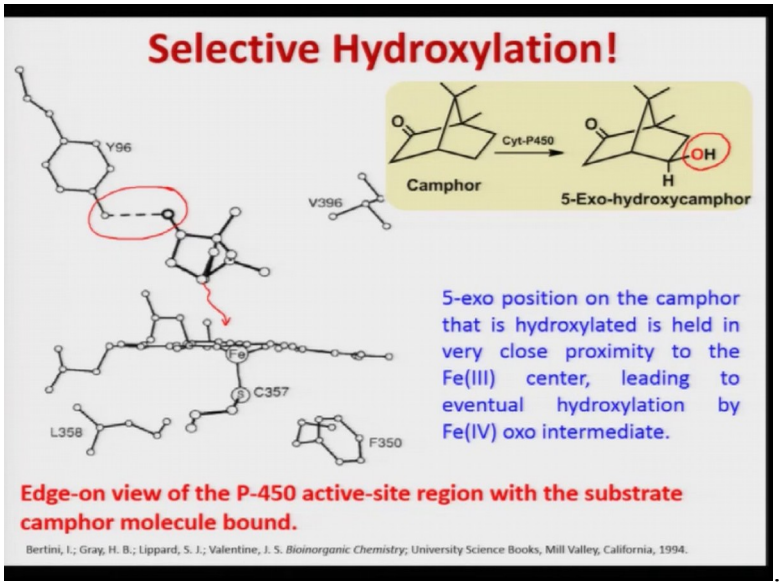

Here actually I have compared the catalytic cycles of catalase, peroxidase and cytochrome P450. As one can see in the catalytic cycle of cytochrome P450, you see this is the resting state of the enzyme which Fe(III)and Fe(III) converts to Fe(II),it is reduced; and then once you become Fe(II), it binds dioxygen and then eventually after this O-O bond cleavage, it forms this Fe(IV)oxo cation radical; will be discussing the catalytic cycle in more details in subsequent lectures.

So, important point is that, that it goes through a highly reactive intermediate which is Fe(IV) oxo cation radical. If you look at the catalytic cycle of peroxidase and catalase, as

you can see that this is once again the resting state of the enzyme; and in both these cases catalase and peroxidase it also going through the same reactive intermediate called compound 1, which is displayed over here that Fe(IV) oxo cation radical.

So, same reactive intermediate it is going through and substrate oxidation taking place.

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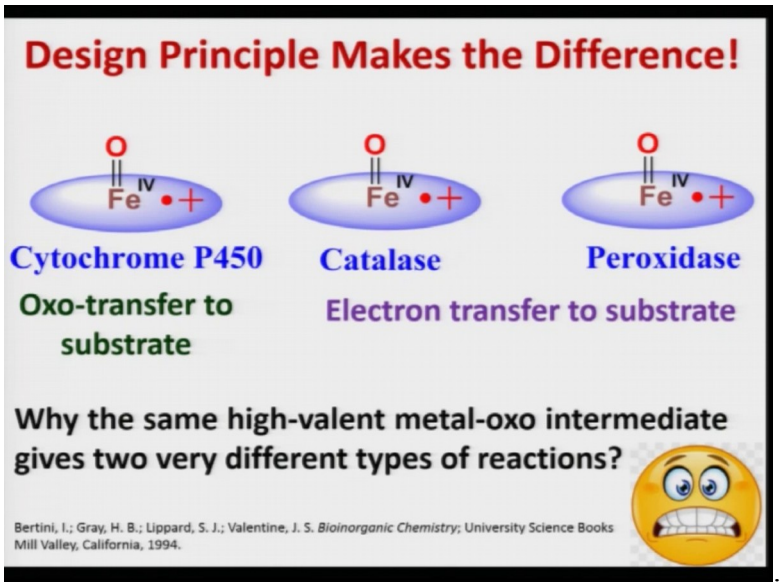

The question is that in cytochrome P450 the oxo-transfer to substrate is what is happening. In contrast, catalase and peroxidase it is the electron transfer to the substrate is happening; although in all these three cases this is the same high valent metal oxo intermediate are present.

So, design principle makes all these difference, which I am going to discuss now.

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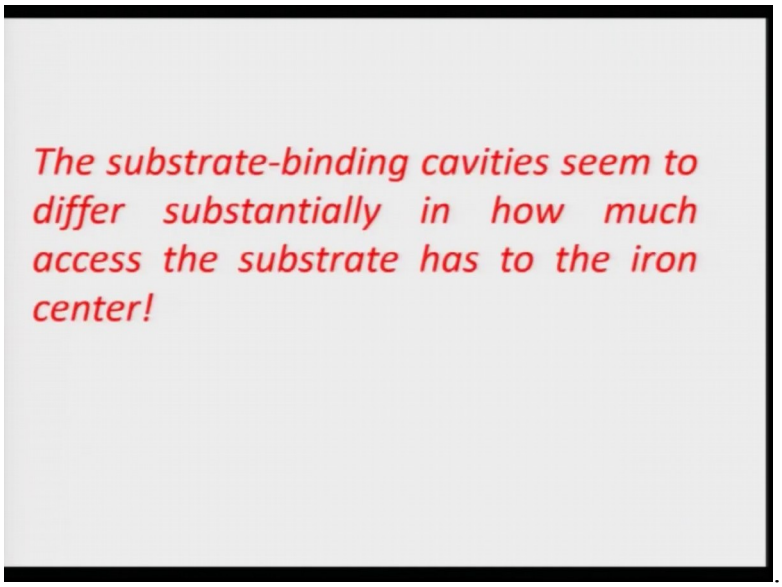

The substrate-binding cavities seem to differ substantially in how much access the substrate has to the iron center.

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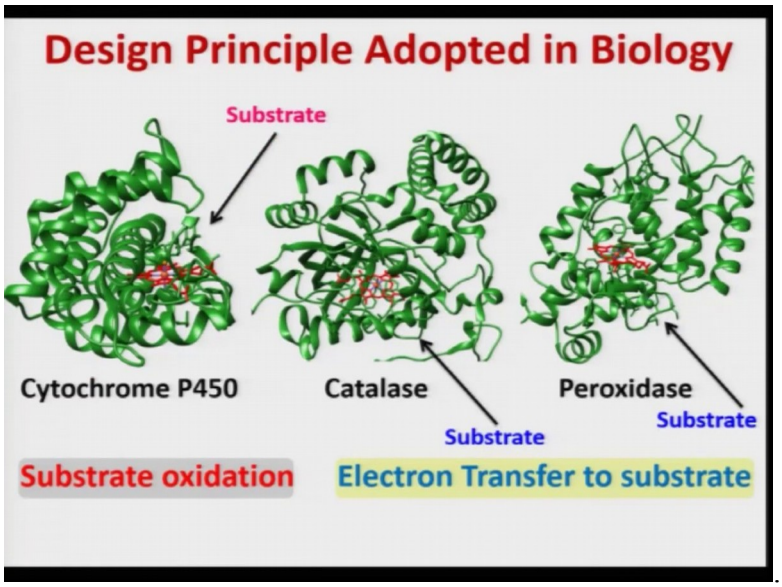

For example the cytochrome P450, catalase and peroxidase structure is shown here. What you see that, the heme center is sitting and the protein chains which are actually wrapping this heme center. In cytochrome P450 there are cavities, where substrate can comes close to the iron center, which we have just observed in the x -raystructure of cytochrome P450 with camphor as a substrate.

In catalase and peroxidase, as one can see that protein is actually covered the entire space on the top of the iron centers and substrate only can come through the porphyrin ring and thereby the electron transfer is taking place. With cytochrome P450 the substrate is jumped right up, against the location where the oxo ligand must reside in the high valent oxo intermediate.

But the same location in the peroxidase enzyme is blocked by the protein structure, so that substrate can interact only with the heme edge, which is displayed over here.

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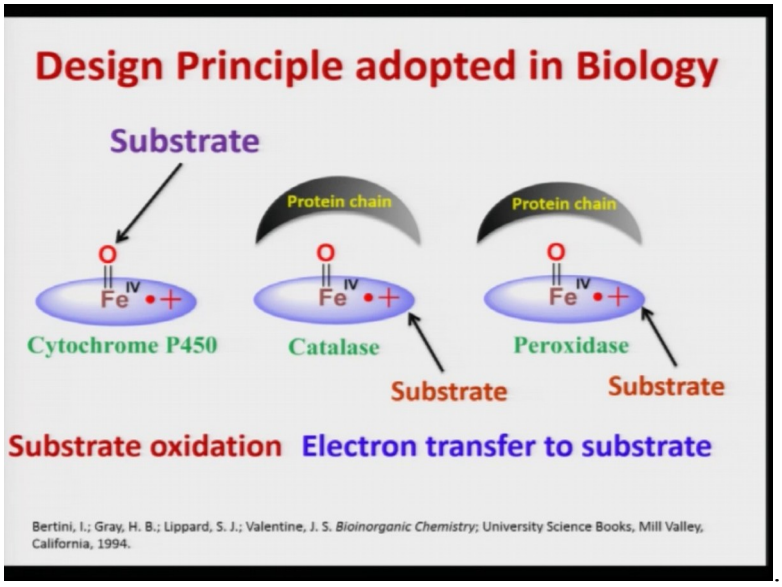

The oxidation of the substrate by electron transfer it is possible for catalase and peroxidase; but the substrate is too far from the oxo ligand for oxygen atom transfer. You see that substrate can come close to this oxo like this; whereas, catalase and peroxidase-substrate can come close to the porphyrin pi cation radical and thereby it is responsible for substrate oxidation in cytochrome P450 and electron transfer to the substrate for catalase and peroxidase.

This is, what is the, beauty of design principle adopted for all these three enzymatic systems; although they all have the same Fe(IV)oxo cation radical, as an active intermediate.

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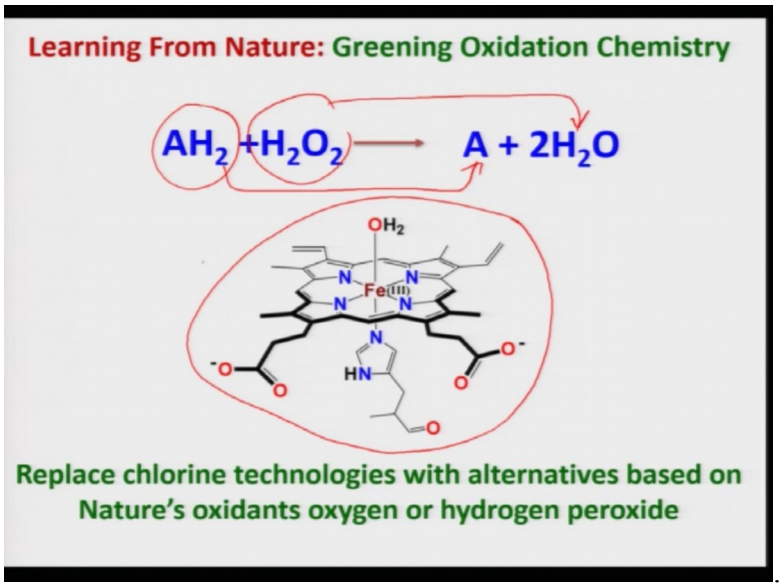

Now, what we can learn from nature, indeed we can learn lots of things while we understand that how this enzyme works. So, indeed that greening oxidation chemistry is possible; say for example, just we have seen in peroxidase, what is happening the substrate is getting oxidized and hydrogen peroxides converted to water.

And this is what is in the resting state of the enzyme; this reaction can indeed replace chlorine technologies with alternatives based on nature's oxidant, oxygen or hydrogen peroxides.

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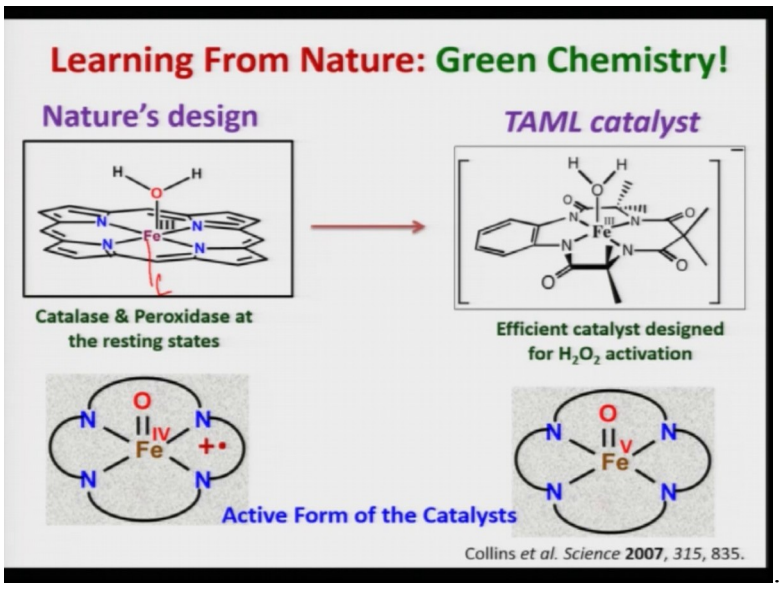

This is the active site structure of catalase and peroxidase in resting state with an axial ligand L and one of our friend from US, Professor Collins, TJ Collins has actually learned and inspired from nature and he made a catalyst called TAML, very similar to whatever is there in catalase and peroxidase active sites.

Only thing is that ligand which actually chelate to the iron is different; although this iron center is also ligated to 4 nitrogen, just like in catalase and peroxidase. So, only difference is instead of this porphyrin as a chelating agent, he used a another chelating agent with keeping everything intact.

So, active form of this catalyst are also very similar as one can see that in catalase and peroxidase; we have just seen this is the Fe(IV) oxo pi cation radical, the ring oxidation actually generates the formation of pi cation radical. However, in case of TAML this is Fe(V) oxo species is the reactive intermediate, they are indeed isoelectronic and they just like catalase and peroxidase are doing lots of chemical transformations in our biological system and this TAML catalyst also being used to do lots of chemical transformations in the laboratory.

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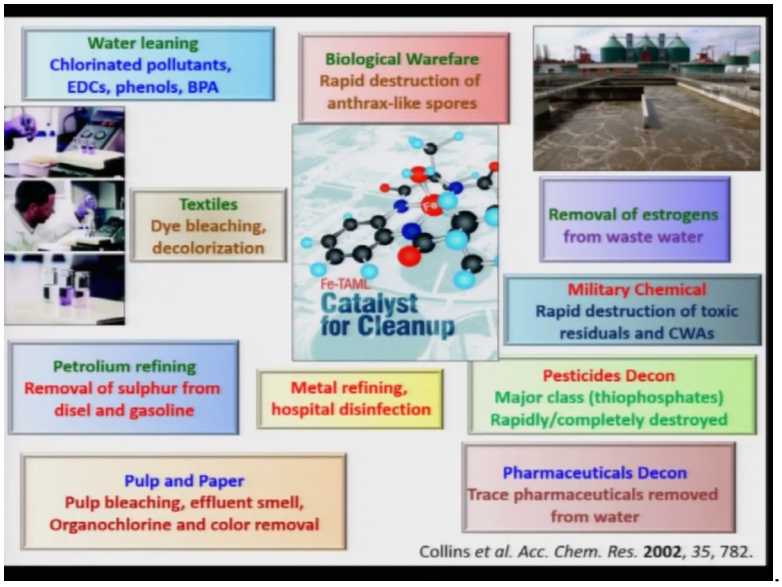

I have shown here, the iron TAML catalyst is being used for various activity in our environment and this is a one of the commercially successful catalyst which is inspired by the enzyme of our mother nature. However, this catalyst as I have discussed, is developed looking at catalase and peroxidase activity in the biological system. Today, I

have showcased how proteins indeed control reactivity in enzymes, although they are all going through a nearly identical reactive intermediate.

Once you understand the mechanism of enzymatic action, we can then implement the knowledge in the laboratory and chemical industry for the betterment of our day to day life. We have provided here, one such example of commercially successful synthetic catalyst, whose design is fully inspired by the enzymes present in our mother nature and have shown it's various practical applications. In my next lecture, I will showcase how by using beautiful design principle, rates of the electron transfer are enhanced by many thousand folds in biology.

Thank you very much.