Bioinorganic Chemistry
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Lecture – 08
Design Principles Used in Chemical Biology: Blue Copper Proteins

Today, we will be talking about the design principles used by nature. We will see the remarkable effect of this design, what we are going to discuss is that why rate of electron transfer is so fast. We will take an example of blue copper protein and we will see soon that how design principle is so important.

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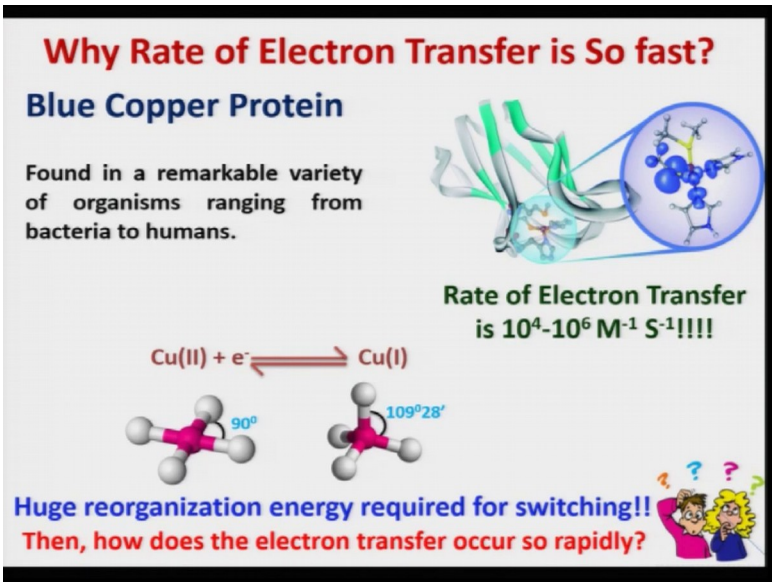

Now, why rate of electron transfer is so fast in blue copper protein? Blue copper protein as you all know found in a remarkable varieties of organisms, ranging from bacteria to human and rate of this electron transfer is very high 10^4 to $10^6 M^{-1}S^{-1}$.

What exactly happening in blue copper protein? Basically, it is inter conversion between Cu(II) and Cu(II). Copper II takes an electron gives rise to Cu(I) and Cu(I) releases one electron and converts to Cu(II). Now for 4 coordination, what you can see that Cu(II) prefers to stabilize in a square planar geometry. In contrast Cu(I) stabilized in a tetrahedral geometry.

Now, this being so, that a huge reorganization energy is required for such switching, square planar to tetrahedral and tetrahedral to square planar. And how does electron transfer occur so fast?

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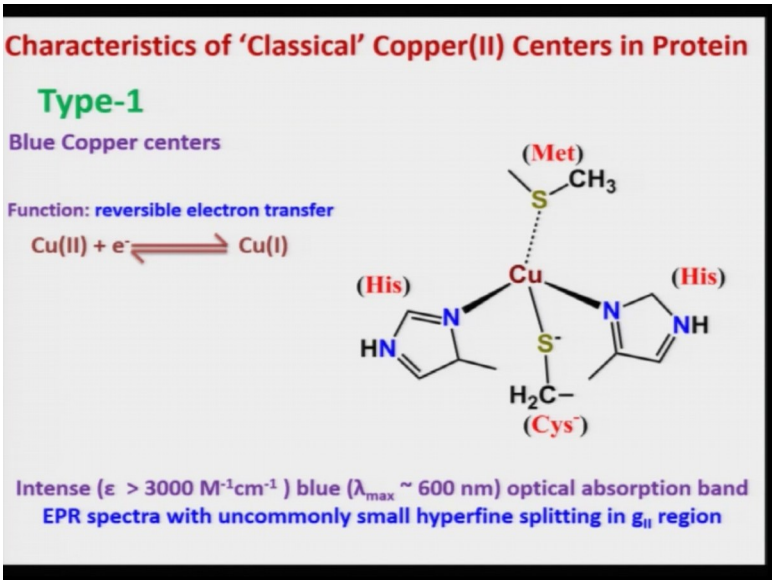

Now, before we discuss about blue copper proteins in details, let us look at the characteristics of classical Cu(II) centers in proteins. There are three types of classical copper centers the type 1, which is called blue copper center. And as you can see here that the function is reversible electron transfer. So, Cu(II) takes one electron becomes Cu(I) and Cu(I) becomes Cu(II).

So, the structure of these blue copper centers in the resting state is shown over here. You can see that copper center is ligated with two histidine one cysteine sulfur and one met sulfur thioether sulfur which is giving in weak copper sulfur bond. Now, in the oxidized state, they have some remarkable features like the they produce intense blue absorbance at nearly 600 nanometer and EPR spectra is uncommonly different from other produce small hyperfine splitting in g parallel region.

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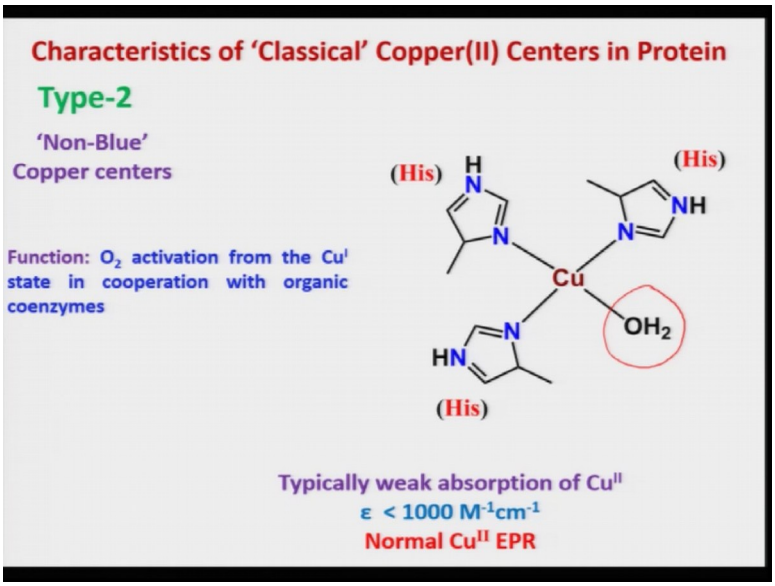

In contrast Cu(II), which is known as non blue copper center, its function is that dioxygen activation from Cu(I) sites in cooperation with organic enzymes. So, if you look at this active site structure is basically this copper center is ligated with 3 histidine and 1 weak water ligand is coordinated at the fourth position. And, the characteristic features of this oxidized species is typically it produce weak absorbance and less than $1000 M^{-1}cm^{-1}$ epsilon value. And, also basically it produce normal Cu(II) EPR.

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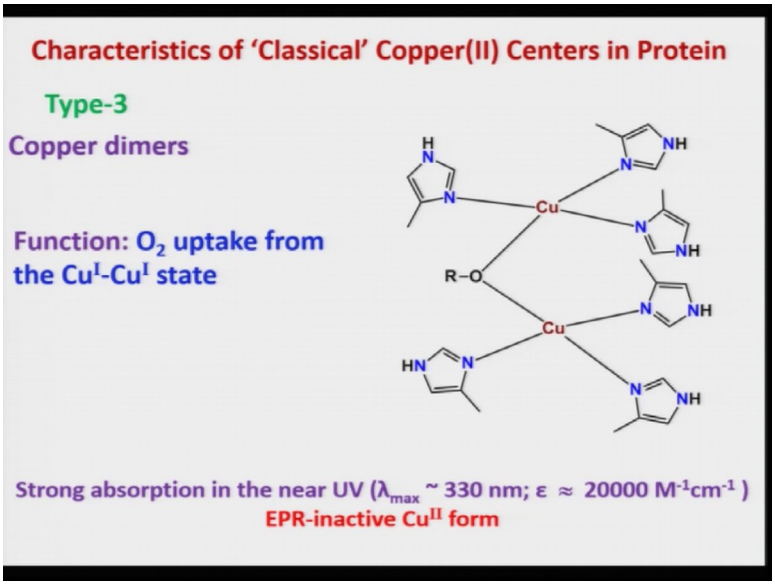

Now, there is another type called type 3 copper dimers. This kind of molecule you see, when you would see that oxygen uptakes from Cu(I)-Cu(I) and this is a dimeric structure and in that oxidized form there is a strong absorption in the near UV region nearly 300 nm. And, because of the strong anti ferromagnetic coupling between two copper centers this Cu(II) are EPR inactive.

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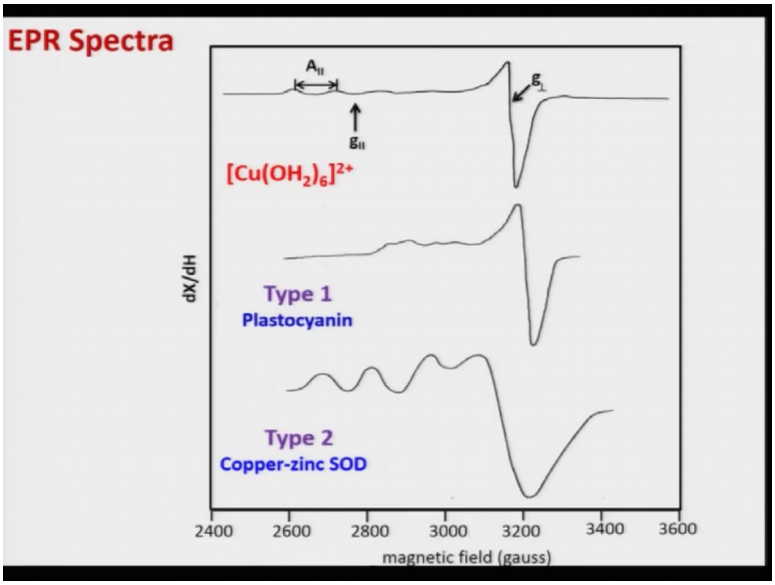

Now, let us come to their EPR spectra as you can see that that copper II hexa aquo species gives rise to an EPR signal as shown as you know that copper's I value is 3/2. So, there will be four lines and because of electron nuclear spin hyperfine interaction denoted by the parameter A anisotropy of the hyperfine interactions is manifested by components parallel and perpendicular to the molecular axis, namely A parallel and perpendicular.

As you can see that this A parallel the separation between these two signal there are four 1 2 3 4 four signal at the parallel and there are perpendicular regions are here and this separation is A parallel. And, if you look at that type 1 and type 2 you see that in type 1 this A parallel is very small. Whereas, in type 2 you see that A parallel is quite large that is normal copper II center, but type 1 which is actually blue copper proteins produces this type 1; you see that very small A parallel value and this is quite interesting and we will talk about that.

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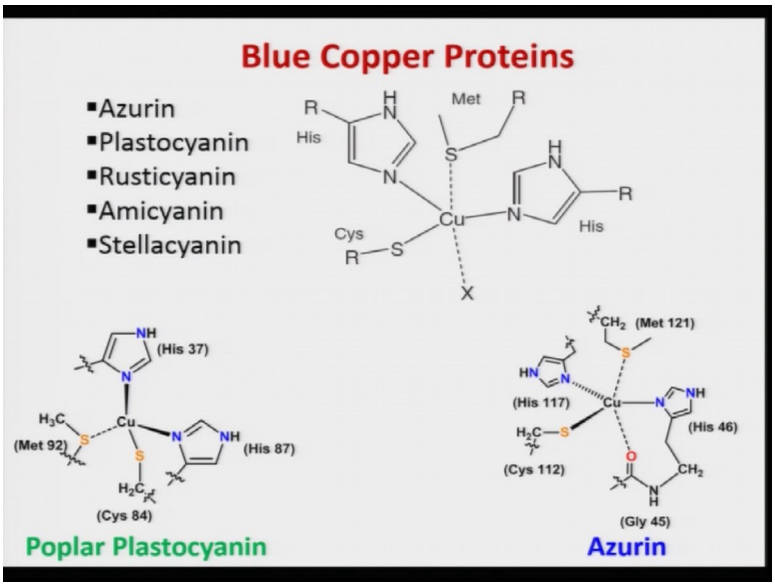

Now, coming back to blue copper proteins, which is large number of proteins are actually comes within this family of blue copper protein. Some of them are azurin, plastocyanin, amicyanin, stellacyanin. And, the active site structure of this blue copper protein is more or less like this as I have said earlier, this copper center is strongly coordinatedwith 2 histidine ligand and 1 cysteine sulfur 1 met sulfur and there is a additional x is also found to be present and this X is some times co-donor from carbonyl group at a distance of 2.8 to 3.2 Å.

Now, two representative blue copper proteins are shown here: one is poplar plastocyanin, 2 histidine are strongly coordinated and then cysteine sulfur also very strongly bound with the copper, but this met sulfur is weakly coordinated. In azurin in contrast you see that it is very similar with the poplar plastocyanin; however, there is an X which is coordinated to carbonyl oxygen and met sulfur is also weakly coordinated. So, these two are weakly coordinated to copper and these 3 bonds are strongly ligated and we will see soon there are spectral features.

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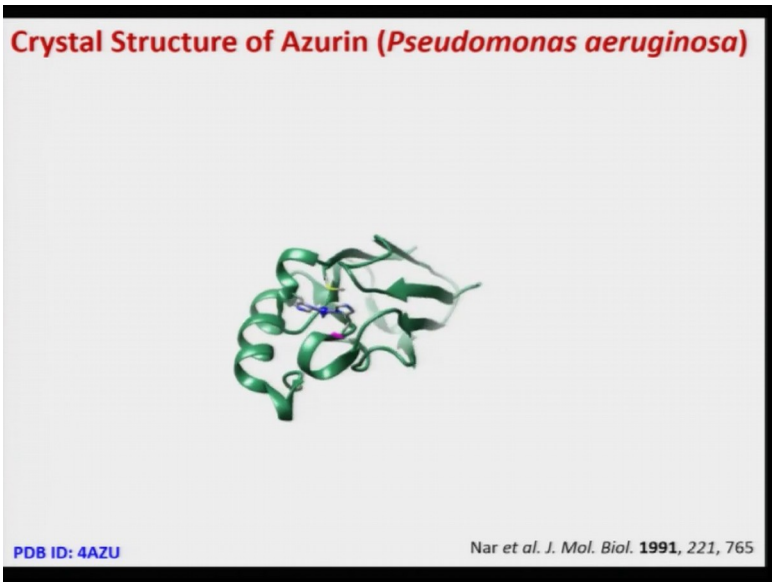

Now, crystal structure of azurin is shown over here. You see there is a huge protein chains which are wrapping around this copper center and as you can see that copper center strongly ligated with 2 histidine nitrogen 1 cysteine sulfur.

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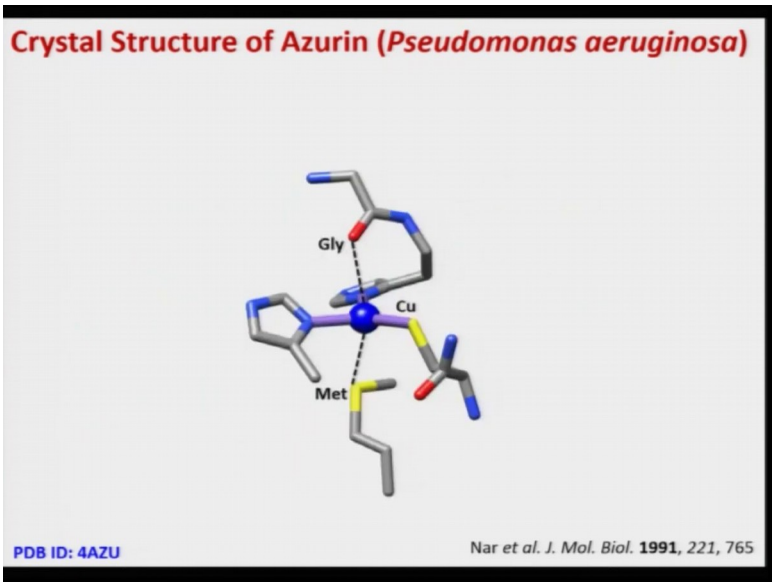

And, this other two are just you know very weak bond. Once we remove this protein you can see very clearly these dotted line are very weak bond in the fourth and fifth position. The crystal structure of poplar plastocyanin is shown here. Here again this copper center is buried within this protein chains.

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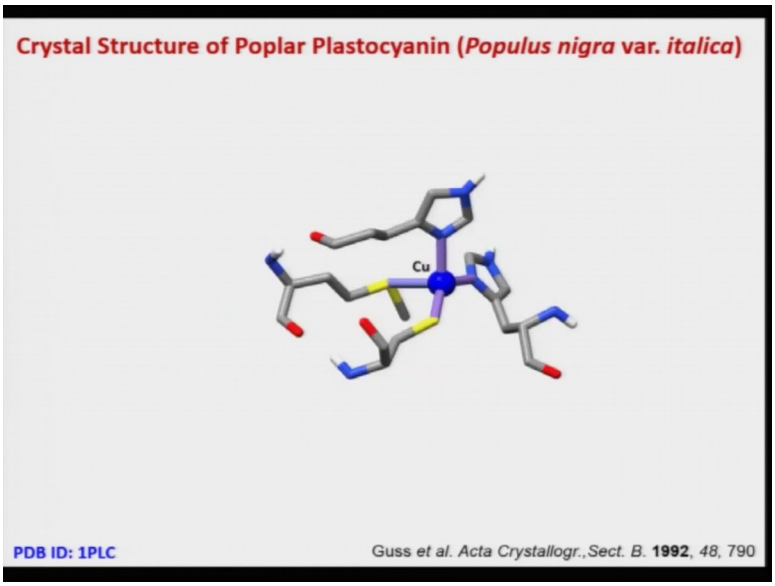

And you can, if you remove this protein then you see this copper centers clearly which is ligated with 2 histidine and 1 cysteine sulfur and 1 met sulfur thioether. And, this is of course, this is a long bond bond is very long. This is the structure of poplar plastocyanin in the resting state.

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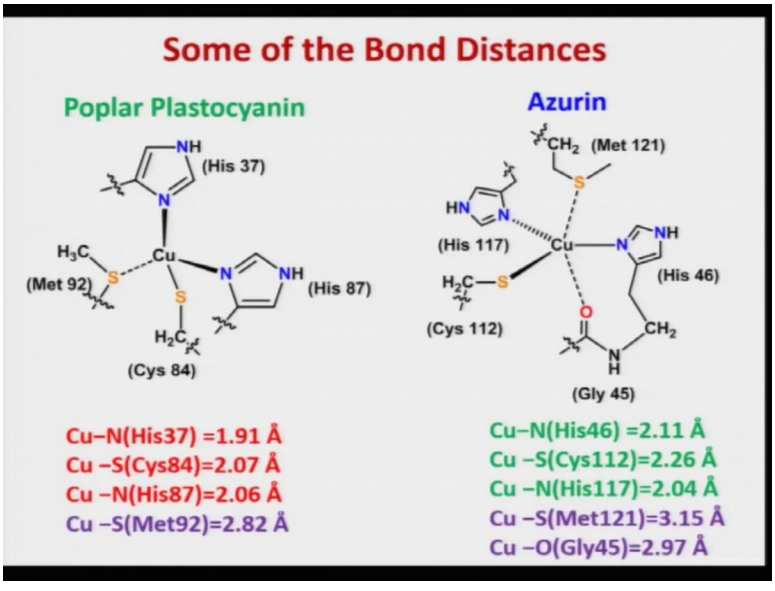

Now, some of these bond distances are shown here as I have just said that, this copper nitrogen histidine nitrogen has strongly bonded 1.91, 2.06 over here. Copper cysteine

sulfur is also strongly ligated 2.07; interestingly copper met sulfur bond is very weak 2.82 Å in poplar plastocyanin.

In contrast azurin also, this copper nitrogen, this histidine to histidine and the cysteine sulfur distances are also very short; that means, they are strongly bonded. And, as I have said earlier that fourth and fifth ligand like copper met sulfur distance is 3.15 very weak distance and copper oxygen is also 2.97 again very weak which is actually obtained in the X-ray structure of azurin.

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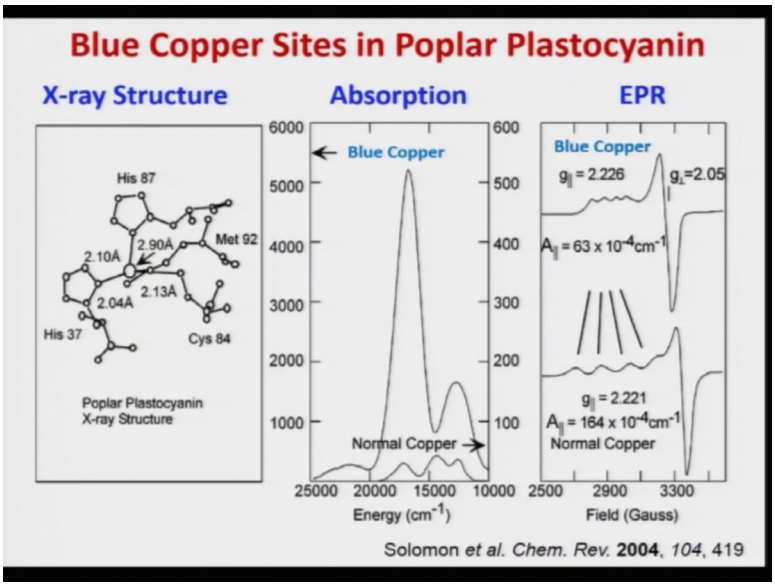

Now, some of these spectral features are shown over here in poplar plastocyanin. X-ray structure is shown here and this is what is the absorption you can see, that the huge difference between blue copper and normal copper and EPR also shown here has been compared with the blue copper protein and normal copper and there is a huge difference.

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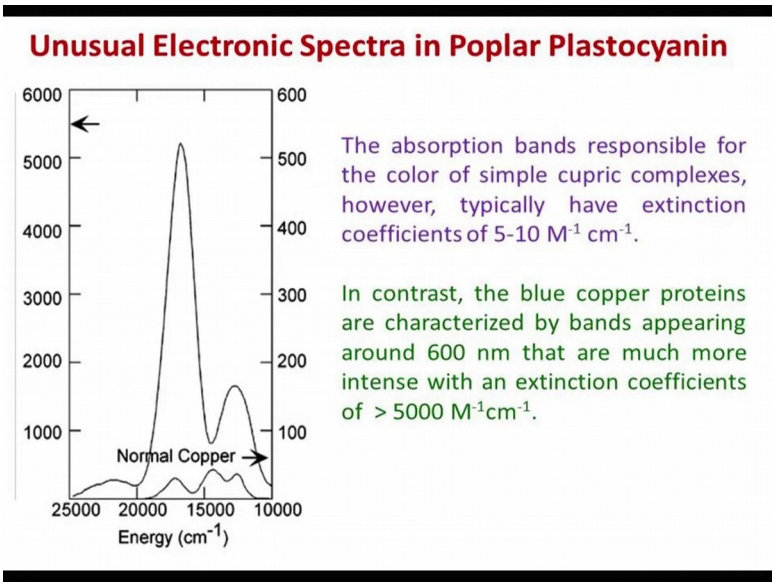

Now, I will discuss one after another like electronic spectra of Cu(II) center in poplar plastocyanin. As you can see this the spectra is compared between normal copper and blue copper protein and the absorption bands actually responsible color of simple cupric complex.

However, typically have extension coefficient of only 5-10$M^{-1}cm^{-1}$ which is clearly reflected as you can see they are all low intense bands. In contrast, what we see in blue copper protein that there is a highly intense band around 600 nm with an extinction coefficient of more than 5000$M^{-1}cm^{-1}$; so, huge spectral change between normal copper and blue copper.

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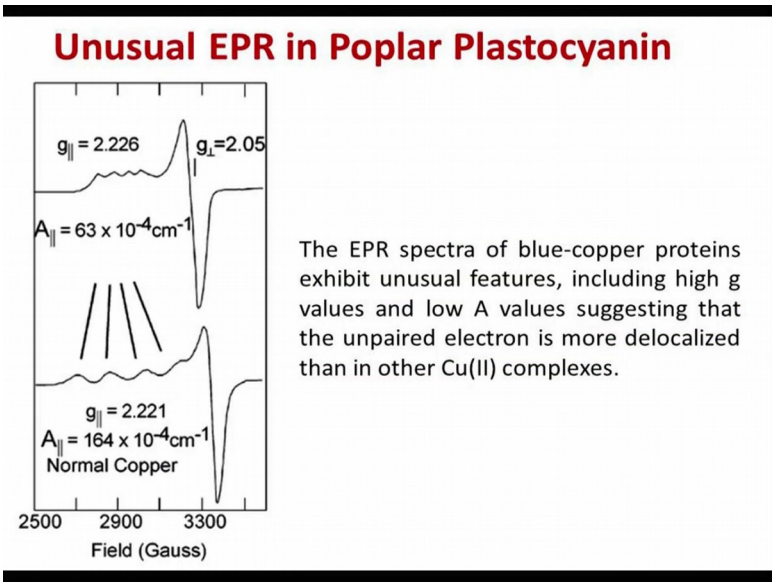

If you look at the EPR spectra of this Cu(II) center; you see that in case of normal copper, this A parallel is a very large 164 x $10^{-4}cm^{-1}$. In contrast in blue copper protein it is only 63 x $10^{-4}cm^{-1}$. So, if your spectra of blue copper proteins exhibits unusual features including high g values and lower A values suggesting that unpaired electron is more delocalized then in other Cu(II) centers.

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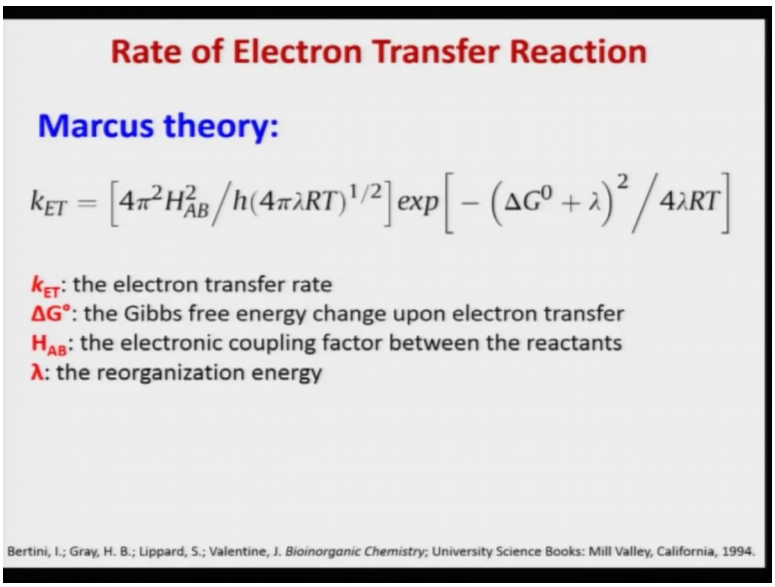

Now, let us little bit talk about rate of electron transfer. Now, as you all know that in Marcus theory this k_{ET} this is the expression and you see that k_{ET} that the electron transfer rate, which is related to H_{AB} which is called electronic coupling factor between the

reactants. Also it depends on ΔG^0, which is the Gibbs free energy change upon electron transfer and one very important factor which is called λ reorganization energy.

Here you see, here you see, the reorganization energy, which is basically described as the energetic cost related to the conformational change between reduced and oxidized species. So, this is the factor, which is actually changes from one molecule to another molecule.

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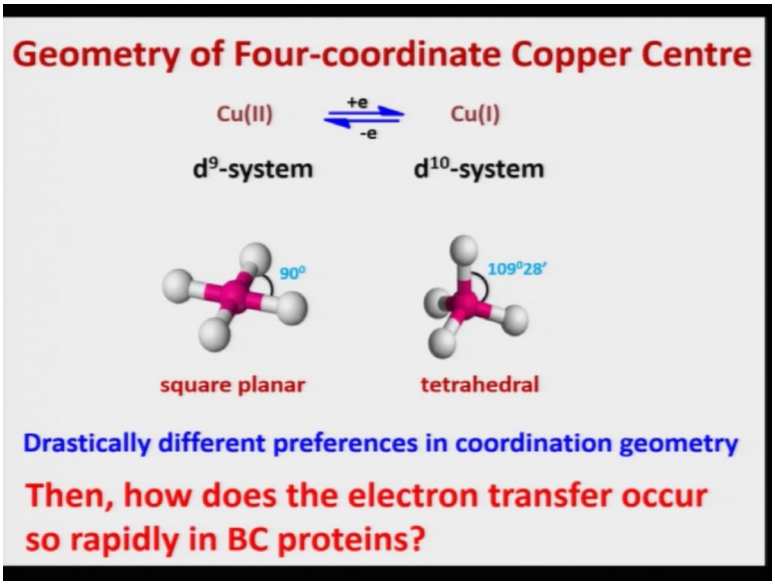

Now, we will talk about the geometry preference of four coordinate copper centers. Like Cu(II) and Cu(I) they are inter converting between them. Now, Cu(II) actually is a d^9 system and Cu(I) is a d^{10} system. Now, d^9 as you know that it prefers to stabilize a square planar geometry whereas, d^{10} prefers as a tetrahedral geometry because then the ligand-ligand repulsion would be minimized.

Now, changing from square planar to tetrahedral and tetrahedral to square planar it is a drastic geometrical change ok. So, there is a drastically different preference in the coordination geometry and if this is so, then how does electron transfer occurs so, rapidly in blue copper proteins.

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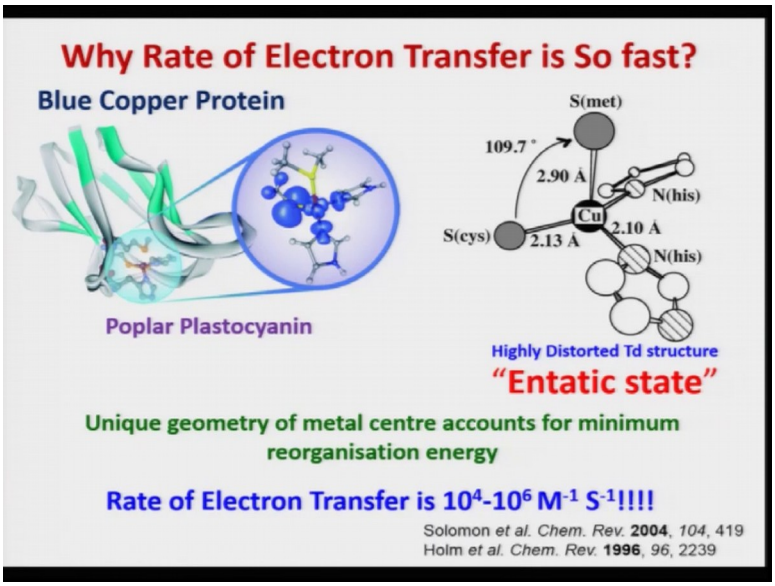

So, blue copper protein structure is shown over here and you can see that this angle cysteine sulfur copper and met sulfur this angle is 109. 7°. Basically, this is a highly distorted tetrahedral structure with a very long copper thioether bond of 2.90 Å. This is high energy molecule, high energy geometry and it has been said that this is in entatic state is high energy state. This unique geometry of metal center accounts for minimum reorganization energy. And that is the reason, why rate of electron transfer is so fast.

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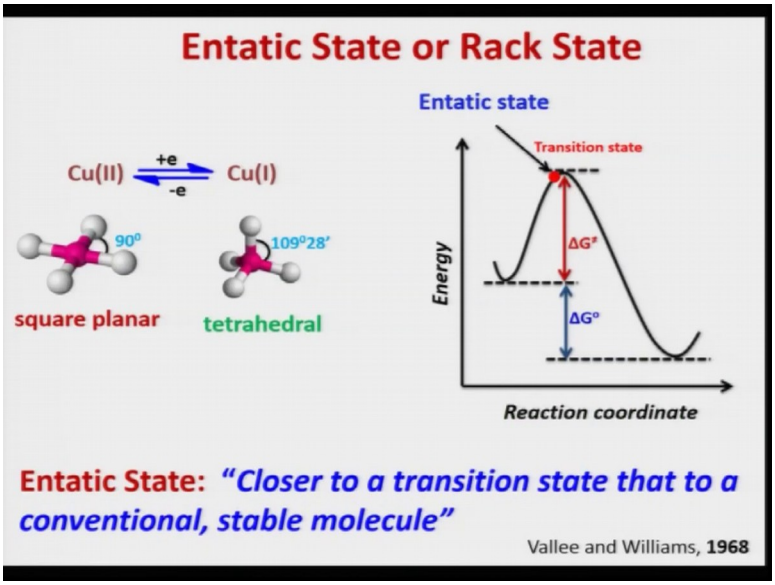

Now, let us talk about Entatic state or Rack state, basically this Cu(II) and Cu(I) they required normally a huge reorganization energy in geometry while transforming between each other. However, in the blue copper protein, we have seen that there the geometry is distorted tetrahedral structure both in Cu(II) and Cu(I) state. Now, this is certainly in a high energy state, which is called entatic state and entatic state has been coined by Vallee and Williams in 1968 which basically they define as a closure to a transition state that way conventional stable molecule.

You can see that that is a hypothetical diagram is shown over here, this molecule is close to the transition state. So, it can go through this direction or the other direction very easily because reorganization energy is very less, it is neither square planar not a pure tetrahedral. So, it is actually a distorted tetrahedral structure and both Cu(I) and Cu(II) have the same geometry.

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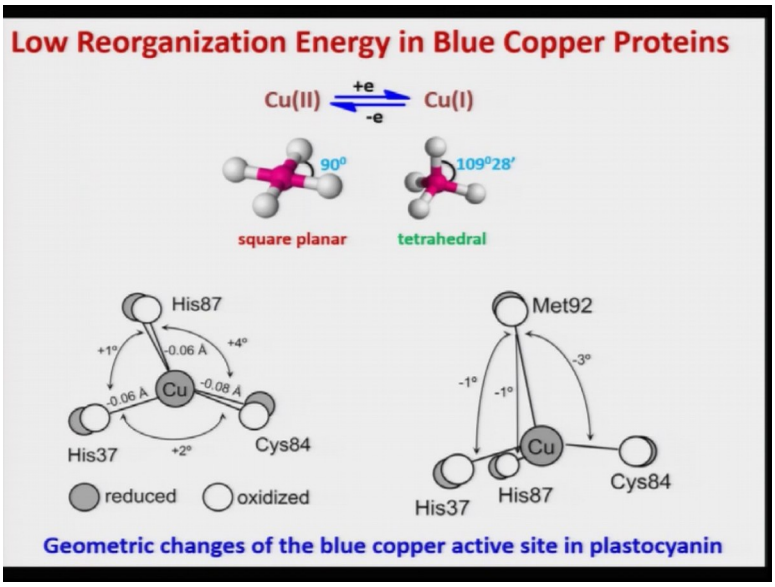

Now, let us look at that, how in entatic state the reorganization energy is reduced? As discussed that Cu(II) and Cu(I) has completely different geometry; however, if you look at the X-ray structure of both oxidized and reduced state of blue copper protein and overlap with one another. So, what you see? You see that there is a very small change between oxidized and reduced state.

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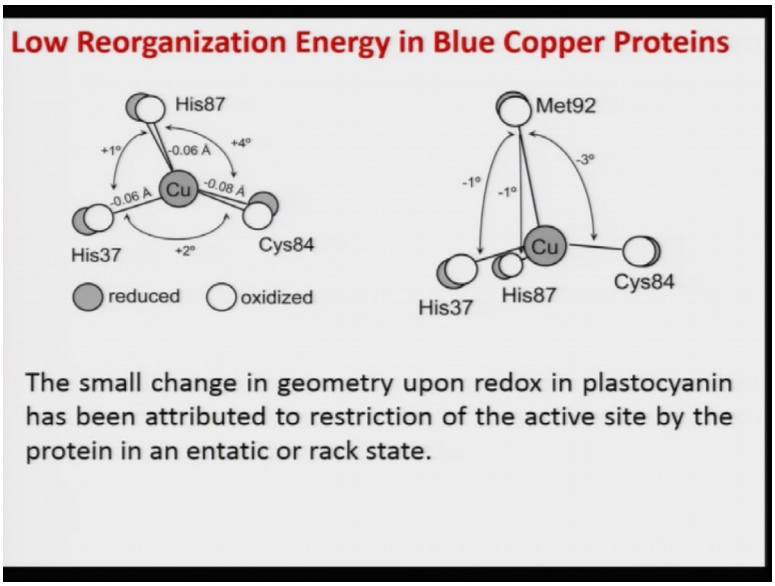

So, the geometry is so rigid that such small change in geometry upon redox in plastocyanin has been attributed to restriction of the active site by the protein in an entatic or rack state. I am showing you once again, the protein structure of poplar plastocyanin, as you can see that all these coordination sites are actually very rigid. And, protein actually creates those rigidity and they stabilize this distorted tetrahedral structure both in oxidized and reduced state.

Now, once you remove, this protein you can see the structure; however, the copper ion can be often be removed from blue copper proteins by the treatment with cyanide. The apoprotein obtained in this manner was crystallized and found to be isomorphous to the metal containing forms indicating that the structure undergo very little change upon metal removal.

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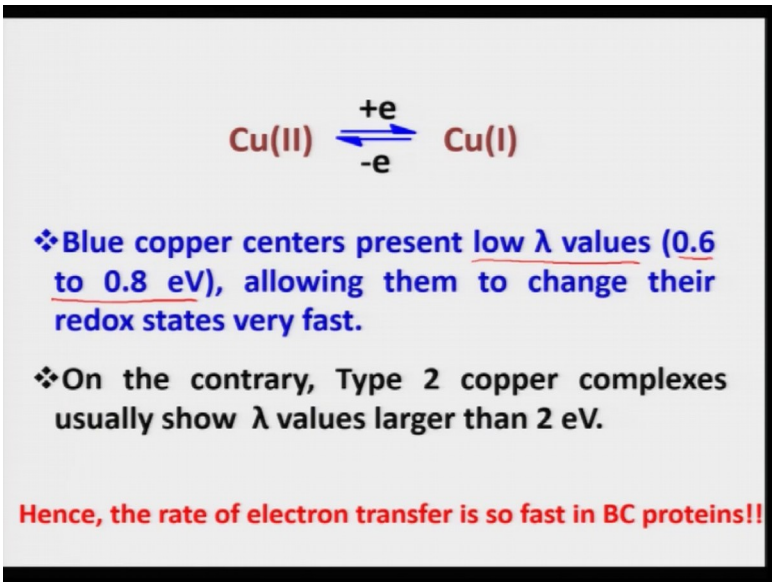

So, this is clearly reflected in the reorganization energy as you can see in blue copper protein; the reorganization energy is very low it is between 0.6 to 0.8 eV and thereby, it allowing to change their redox state very fast. In contrast type 2 copper complexes usually so large reorganization energy sometimes larger than 2 eV. Hence, rate of the electron transfer is so fast in blue copper proteins.

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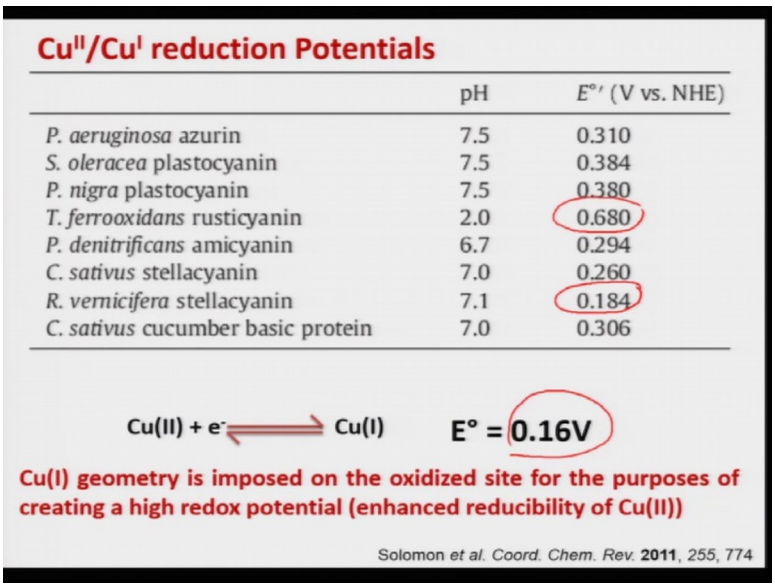

Now, Cu(II)/Cu(I) redox potentials are shown here; as you can see that, these are the blue copper proteins isolated from different sources and under physiological pH they

varied their potential remarkably from 0.184 to 0.680 V. Please note that Cu(II)/Cu(I) redox potential is only 0.16. So, it is high positive value in the blue copper proteins and this is because Cu(I) geometry is imposed on the oxidized site of for the purpose of achieving a high redox potential.

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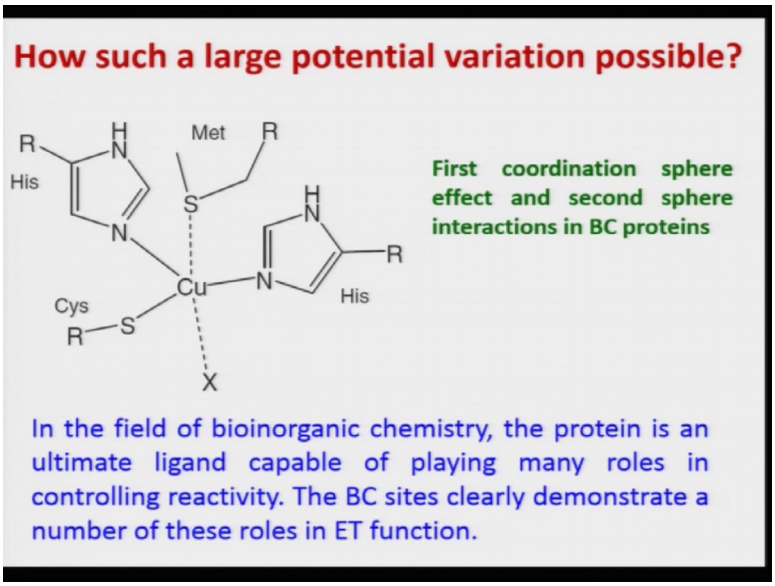

Now, next question is that, how such a large potential variation is possible? So, this is basically this active site structure of blue copper protein. First coordination sphere also effects and it can changes this redox potential along with the second sphere interactions from the protein. Indeed both are contributing towards such a large variation in redox potential value. It is interesting to see here, in the field of bioinorganic chemistry the protein is an ultimate ligand capable of playing many roles in controlling reactivity. The blue copper sights clearly demonstrate in number of these roles in electron transfer function.

Today, I have showcased how the rate of electron transfer is enhanced by many thousand folds in blue copper proteins as compared to the normal copper centers. The unique spectroscopic features of the blue copper proteins are clear reflections of the role of protein chains in stabilizing the unusual geometric and electronic structure of blue copper site for its function; I also have illustrated here, how such a unique geometry in both the oxidized and reduced states of copper accounts for the minimum reorganization

energy and thereby enhanced rate of the electron transfer. The concept of the entatic or rack induced state has also been discussed here.

Thank you.