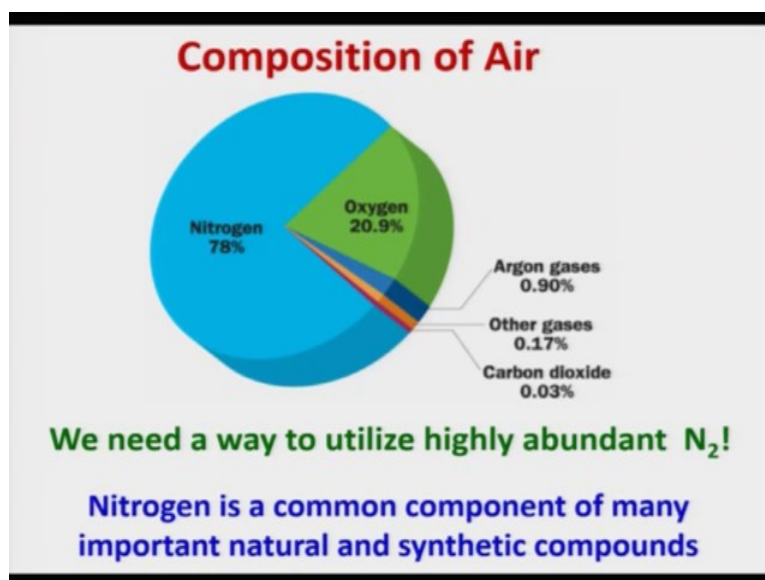


Bioinorganic Chemistry
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Indian Institute of Technology, Kanpur

Lecture – 09
Design Principles Used in Chemical Biology: Nitrogenase

We have been talking about the Design Principle Used by Nature and we have seen in the past the beauty of this design and how the design makes all the difference in reactivity, in rate of the electron transfer and other aspects. Today, we will be looking at some of these very interesting aspects like fixation of nitrogen from air.

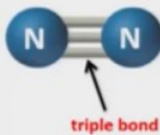
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If you look at the composition of air what you find? You find that 78 % is nitrogen and 20.9 % is oxygen and rest other gases like argon only 0.9 %, carbon dioxides 0.03 and other gases are 0.17 %. So, nitrogen is present in 78 %. And can we utilize nitrogen? Indeed, nitrogen is a common compound of many important natural and synthetic compounds.

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How Inert is the Nitrogen Gas?



Bond Dissociation Energy: 225 kcal/mole

- Very high bond dissociation energy
- Carrying out reactions “under nitrogen”: Inert Atmosphere

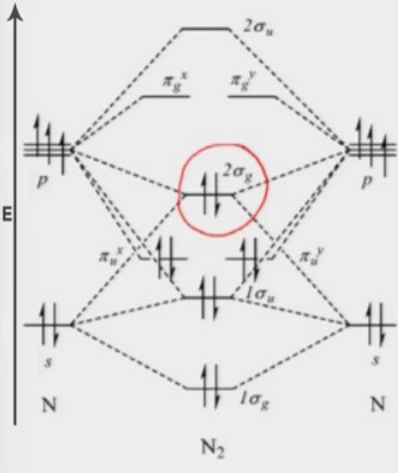
Activation of N₂ seems like a difficult task!



Now, as you all know nitrogen is inert, now how inert is this nitrogen? Now, if you look at this bond dissociation energy it is 225 kcal per mole, it is a huge amount. So, very high bond dissociation energy. Indeed in the laboratory we carry out reactions under nitrogen assuming that they give an inert environment. So, it is so inert. So, we treat them like inert gas. So, looks like the activation of this dinitrogen is a difficult task.

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Molecular Orbital Diagram of N₂



Bond Order = $\frac{6-0}{2} = 3$

Inert

Oxidation and reduction looks like a difficult task!

Now, let us look at the molecular orbital diagram of dinitrogen. As you can see that highest occupied molecular orbital which is a σ orbital contains 2 electrons, it is a bonding

orbital. Now, if you calculate the bond order it is 3, very high bond order. So, that is the reason why it is so inert.

However, if one like to oxidized or reduced in any chemical reaction what would happen? Say, if we like to oxidize then he has to remove that one electron from a bonding orbital which is sigma, so, it is very difficult task. But, if dinitrogen likes to be reduced what would happen? So, electron have to go to π^* anti-bonding orbital. So, which is again difficult. So, removing an electron is difficult adding an electron is also equally difficult. So, that means, oxidations and reduction looks like a difficult task.

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Selected Molecular Properties:

	ΔH_f° (kJ mol ⁻¹)	Proton affinity ^a (kJ mol ⁻¹)	Electron affinity (eV)	Ionization potential (eV)	HOMO-LUMO gap (eV)
N ₂	—	493.8	-1.903	15.841	10.82
N ₂ H ₂	212.9	803			
N ₂ H ₄	95.35	853.2			7.444 ^a
NH ₃	-45.9	853.6	-2.302	10.83	7.75
HC≡CH	226.73	641.4	-1.435	11.28	8.47
CO	-110.53	594	-1.283	14.23	9.34
CH ₄	-74.87	543.5	-1.906	14.18	11.2

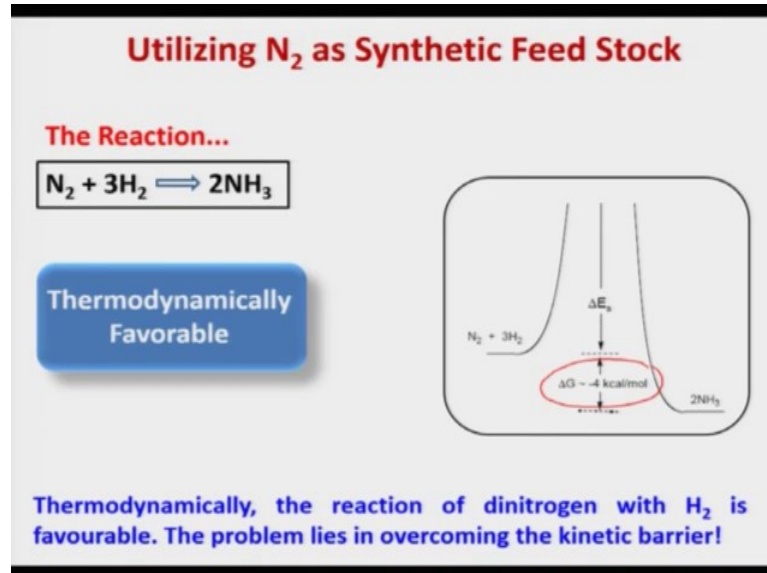
^a Data from the NIST Chemistry WebBook (www.webbook.nist.gov).

This has been reflected in some of this molecular property as shown over here. This ΔH_f° is a standard enthalpy of formation, proton affinity, electron affinity, ionization potential and HOMO-LUMO gap. If you look at the table along with nitrogen there are many other gas or gaseous molecular shown here – like acetylene, carbon monoxide, methane, ammonia, hydrazine all these have been compared.

Now, what you see that this HOMO-LUMO gap is too high 10.82 eV that actually explains that why it is so inert and if you look at this proton affinity what you find that it is the lowest in the series; that means, it is extremely difficult to protonate at even with a strong acid it is impossible task. And if you look at this electron affinity this is large negative. Ionization potential values very high positive. So, these are the indicator that

why dinitrogen is so inert. So, like other factors such as molecule's non-polarity it is high ionization potential and negative electron affinity determines nitrogen inertness.

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Now, let us look at that the most important chemical reactions which is the conversion of nitrogen and hydrogen gives rise to ammonia. So, if you look at this what you see that ΔG is around 4 kcal per mole; that means, it is a negative, that means, it is thermodynamically highly favorable reactions. If this being so, then what is the problem? The problem is also the reaction is thermodynamically favorable, but the problem lies in overcoming the kinetic barrier. You just mix it, it is impossible to get ammonia.

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How Inert is the Nitrogen Gas?

$N_2 + 3H_2 \rightarrow 2NH_3$ $\Delta G^\circ = -3.97$ kcal/mole **Thermodynamically Favorable**

At pH= 7, The Reaction is...

$N_2 + 8H^+ + 6e^- \rightarrow 2NH_4^+$ $\Delta E^{\circ'} = -280$ mV

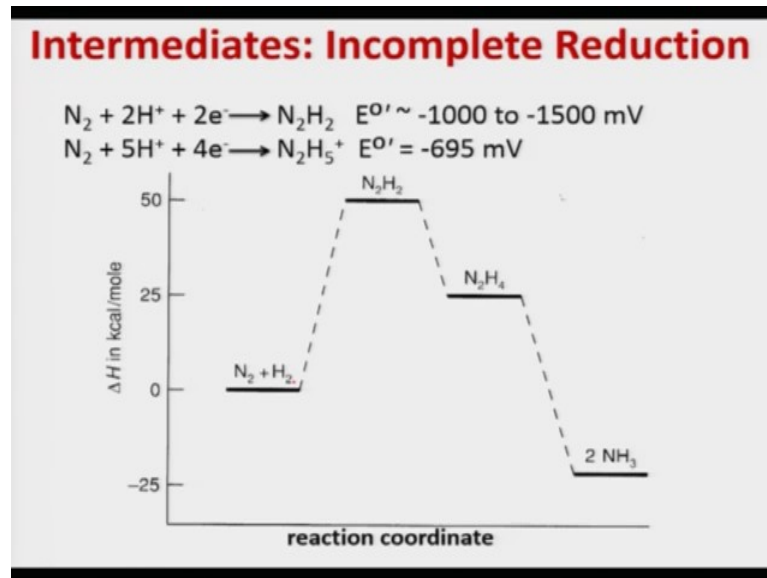
Then, what is the cause of kinetic inertness of N_2 ?

It would require $6e^-$ and $6H^+$ in a concerted process!

So, the question is that how inert is this nitrogen gas as we have just seen that nitrogen plus 3 molecule of hydrogen gives rise to 2 molecule of ammonia which gives ΔG° large negative values. So, it is thermodynamically favorable. However, at pH 7 the reduction the nitrogen plus 8 proton plus 6 electron giving rise to $2NH_4^+$ the $E^{\circ'}$ the formal potential is only minus 280 mV. So, this is a feasible reactions in the biological system, this is not too negative.

So, if this is the case, then what is the cause of its kinetic inertness? As you can see this reactions nitrogen converting to ammonia it requires 6 electron and 6 proton in a concerted process you have to add 6 protons and 6 electron all at a time, then and then only this nitrogen can convert to ammonia.

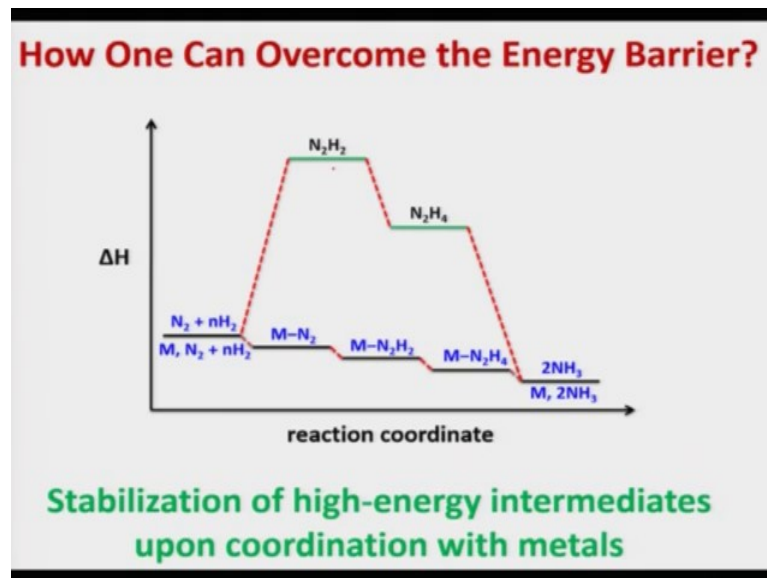
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If you cannot give 6 proton and 6 electron in a concerted way then you end up with intermediates and as you can see here that N_2 converting to N_2H_2 and N_2 converting to N_2H_5^+ . If you look at the formal potentials are very large negative value which is impossible at the biological system; this large potential like minus 1000 to 1500 mV for N_2 to N_2H_2 . And for to N_2H_5^+ this is -695mV which is a very large negative potential and impossible in the biological system to generate this.

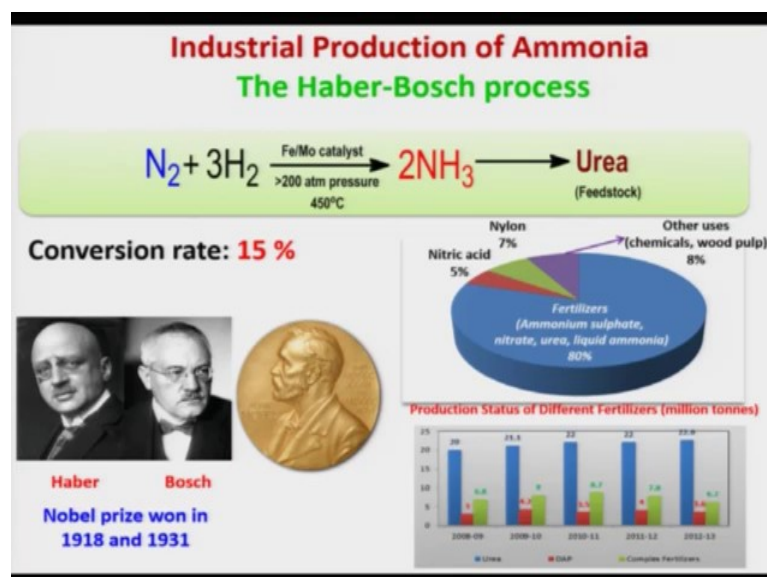
Now, if you look at the diagram what you see that N_2H_2 and N_2H_4 are highly unfavourable because of ΔG is large positive values and looks like it is an impossible task to go through this all this intermediate to reach ammonia. So, we see that if one can give 6 proton and 6 electron all at a time then N_2 plus H_2 can give rise to ammonia. However, it is difficult if it is not all at a time, then it will go through highly unfavourable intermediates and then this nitrogen and hydrogen converting to ammonia is an impossible task.

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Now, how one can overcome such high energy barrier? Yes, it is possible. This energy barrier of this intermediate formations can be reduced drastically by the addition of metal, indeed this coordination with a metal reduces this energy barrier and then you can see that $M-N_2$, $M-N_2H_2$ and $M-N_2H_4$, all these intermediates are highly favorable situation and it can leads to the formation of ammonia. Indeed biology has taken this path, so that this formation of ammonia becomes easier, we will see in details.

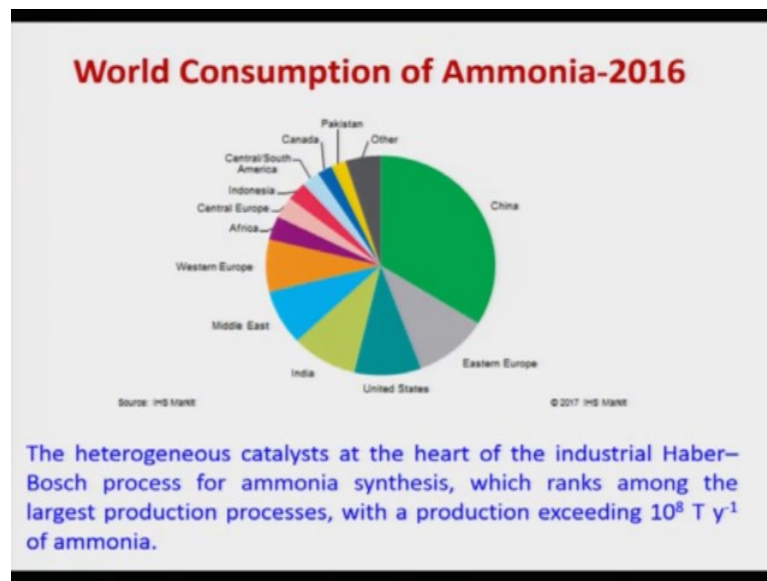
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Now, what happens in industry? So, industrial production of ammonia which is basically nitrogen plus 3 molecule of hydrogen gives rise to 2 molecule of ammonia which eventually converts to urea. If you look at the conditions of this conversion is iron molybdenum catalyst and more than 200 atmospheric pressure and very high temperature like around 450°C and this produces only the conversion rate around 15 percent.

This is indeed the Haber-Bosch process and this conversion is so important that they were awarded Nobel Prize of course, in two different time. Haber got Nobel Prize in 1918, Bosch got the Nobel Prize in 1931 improving the conditions for the formation of ammonia. This formation of ammonia which eventually can be converted to urea is very important for our farmers because this urea is one of the very important fertilizers used for agriculture. So, not only this urea indeed ammonium sulfate, nitrate and liquid ammonia all around 80 percent would be nitrogenous ammonia related products and there are 5 percent nitric acid, 7 percent nylon and other uses also there.

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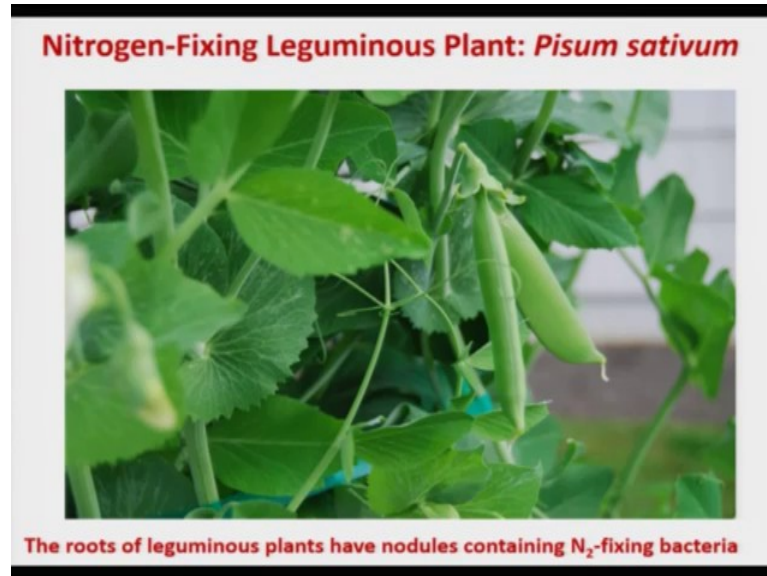


Now, if you look at the world consumption of ammonia you see that China use is consuming a huge amount of ammonia followed by United States, India and European countries, all countries, all farmers utilize this ammonia and they actually produce lots of nitrogenous fertilizer for their agriculture.

The heterogeneous catalysts at the heart of the industrial Haber-Bosch process for ammonia synthesis which ranks among the largest production process, with a production

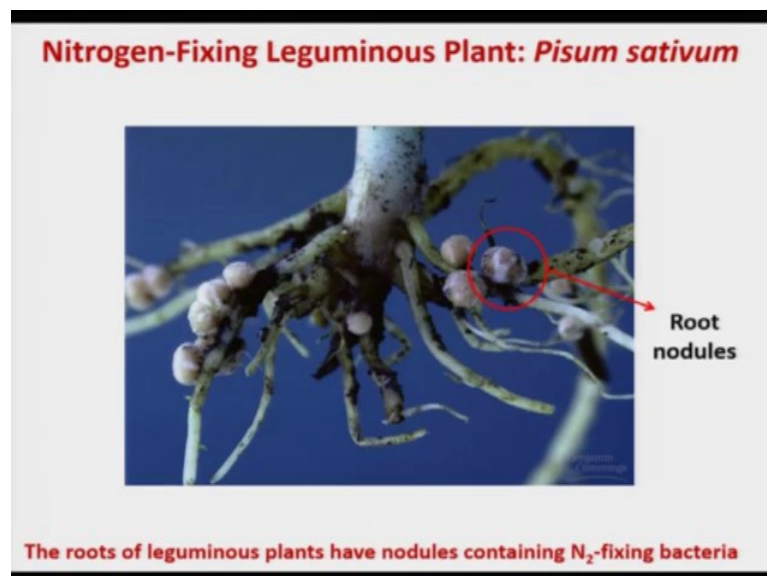
exceeding 10 to the power 8 tons per year of ammonia. So, you see that formation of ammonia is so important for us.

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However, nature has designed in such a way that there are few plants we call it leguminous plant they fix the aerial nitrogen very easily.

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And, you all seen this root nodules where are there is an enzyme called nitrogenase is present which fixes aerial nitrogen to ammonia at room temperature and normal atmospheric pressure.

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Nitrogenase: The Wonder Enzyme


The Reaction...

• Nitrogen Fixation

$$\text{N}_2 + 8\text{e}^- + 8\text{H}^+ \longrightarrow 2\text{NH}_3 + \text{H}_2$$

At room temperature and atmospheric pressure

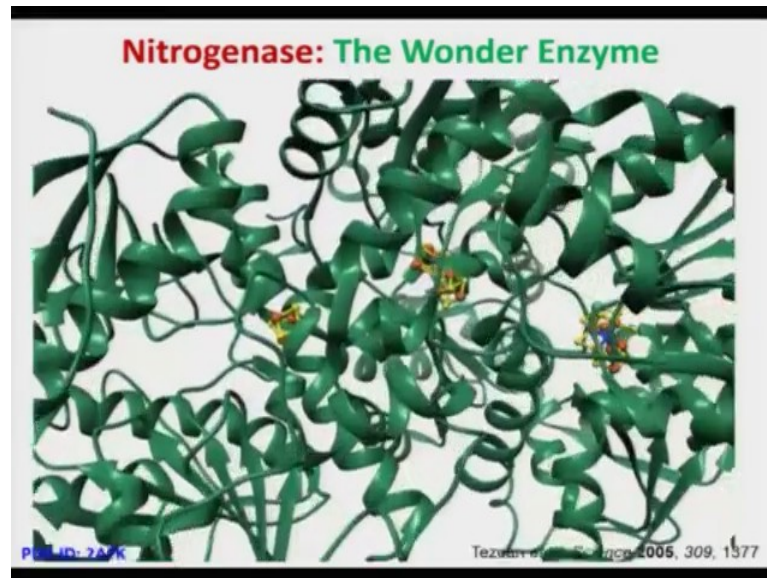
The nitrogenase enzymes, responsible for the atmospheric fixation of about 10^8 T y^{-1} of N_2 to ammonia, necessary for the biosynthesis of ubiquitous nitrogenated natural compounds such as amino acids and nucleic acids.



And, this is very important. So, nitrogenase is the enzyme who does all this miracle in biology. Now, the reaction is N_2 plus 8 electron and 8 proton produces 2 molecule of ammonia plus hydrogen. And, this reaction is happening at room temperature and normal atmospheric pressure and this is what is happening because of nitrogenase enzyme and we like to understand that how nature is actually fixing this aerial nitrogen so easily.

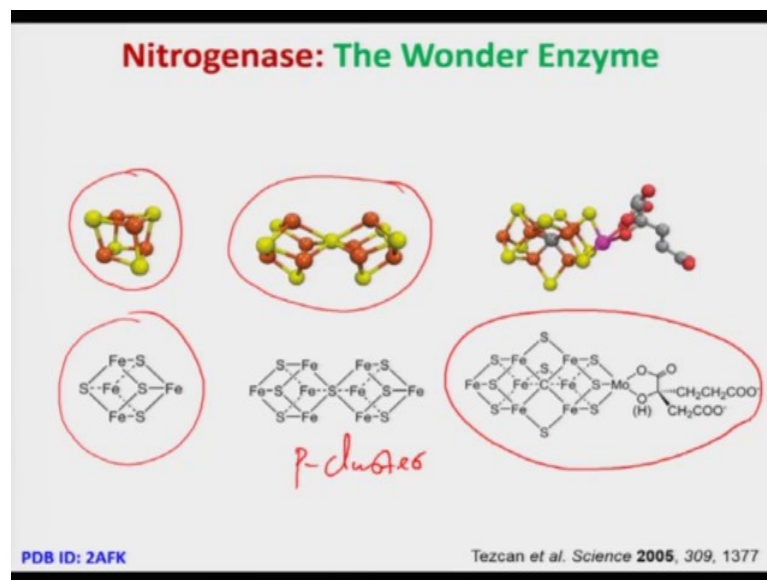
So, nitrogenase enzymes, that responsible for the atmospheric fixation of about 10 to the power 8 ton per year of nitrogen to ammonia, necessary for the biosynthesis of nitrogenated natural compound such as amino acids and nucleic acids. You see that how important is this for our biological system.

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So, this is the X-ray structure of nitrogenase enzyme and as one can see that there is huge protein chains which are wrapping around this molecules.

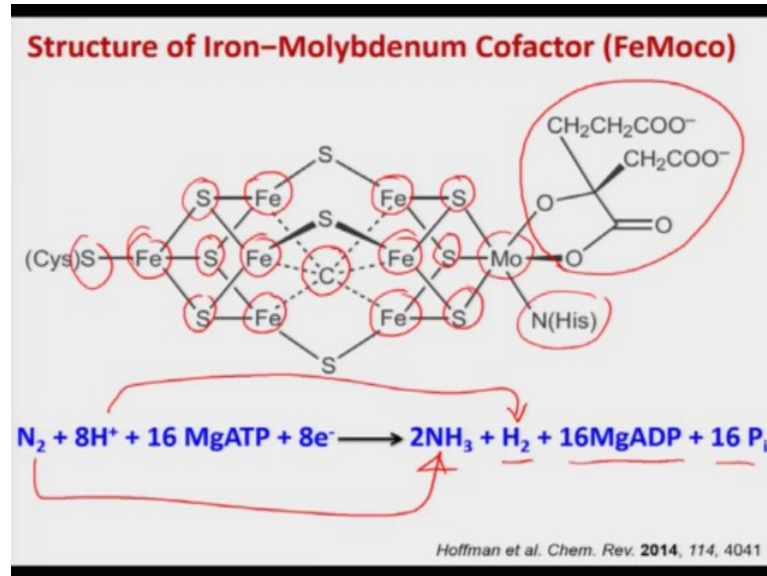
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And, if I remove this protein chain you can see that are coming that metal cluster that are coming immediately iron sulfur cluster over here. And you can see the structure in the chemdraw I have shown over here. Then, this is also similar to iron-sulfur cluster, but not exactly identical this is we call it as a P-cluster and this is that Fe-Mo cofactor. The

structure is shown over here and we now know exactly how this cofactor actually looks like.

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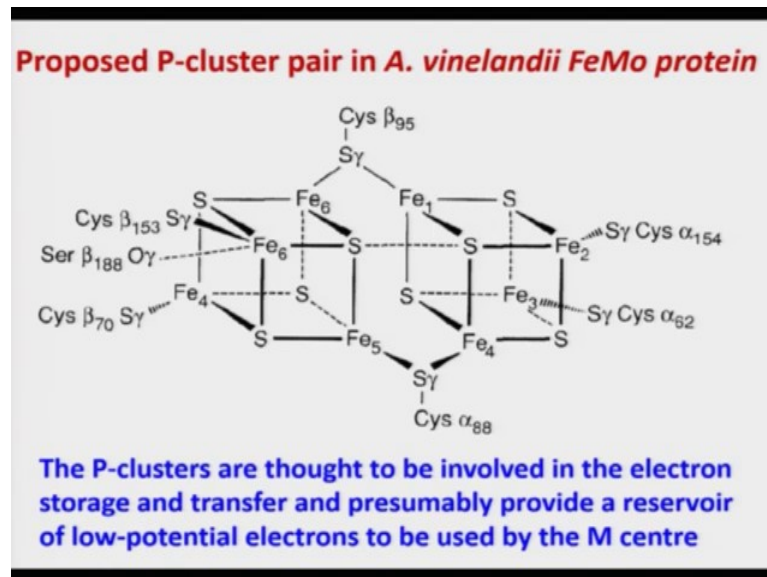
So, this is what is the structure of Fe-Mo factor and there this is the active sites, where this nitrogen is converting to ammonia. Crystallographic studies spanning over 30 years, have led to the current knowledge of this Fe-Mo cofactor structure in its resting state. It is as you can see is a cage like arrangement of 1 molybdenum over here and 7 iron centers 1, 2, 3, 4, 5, 6, and 7 iron centers and a central non-exchangeable carbon single atoms in the central cavity of the case during nitrogen reduction.

This coordinatively saturated molybdenum center is saturated by three sulfide 1, 2, 3 three sulfide and 1 nitrogen from histidine and also a unusual bidentate homo citrate ion. You see this is the homo-citrate ion. It is coordinating in this bidentate fashion to molybdenum in the crystal structure of the resting state no available coordination sites for dinitrogen unit is present around the molybdenum center.

One of the 7 iron center the one most distant from the molybdenum atom of the cofactor is tetragonally coordinated to three bridging sulfide group 1, 2, 3 and 1 cysteine sulfur. The remaining 6 central iron atoms are in tetragonal coordination, three ligands being sulfur bridges, the fourth being the carbon atom. The 6 central iron atom constitute three open Fe_4S_4 faces and as I have said that this iron molybdenum cofactor is responsible for this conversion of nitrogen to ammonia.

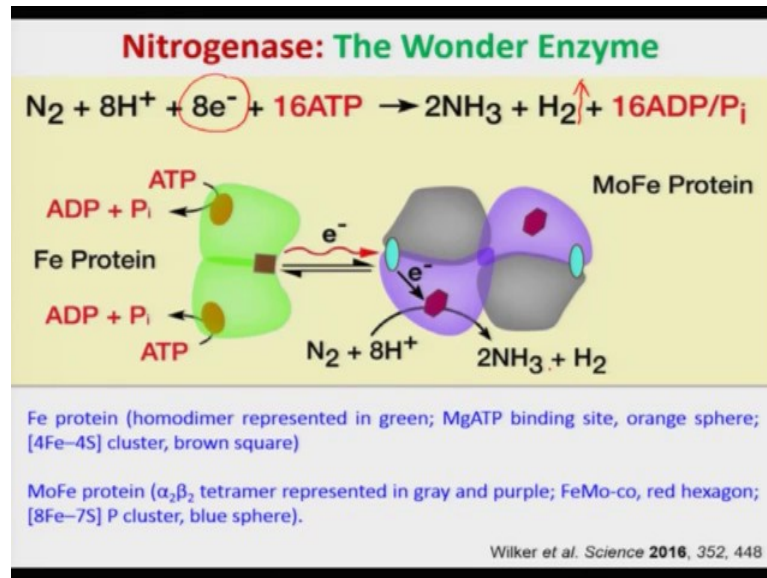
You can see that N_2 plus 8 H plus 16Mg-ATP plus 8 electron gives rise to 2 molecule of ammonia and a H_2 and 16Mg-ADP plus P i. ATP is giving the energy huge amount of energy is required is provided by ATP converting to ADP and giving rise to this P i phosphate and this reaction also helps maintaining anhydrous atmosphere and appropriate pH at which the enzyme is most active. So, all these reactions are covered and this is very important. So, along with reduction of nitrogen to ammonia you see that one molecule of hydrogen also produced during this transformations.

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Now, this is the proposed P-clusters structure. It is not exactly iron sulfur cluster. As you can see that there are 2 iron sulfur cluster connected with the cysteine sulfur and this P-cluster or thought to be involved in the electron storage and transfer and presumably provide a reservoir of low potential electrons that to be used by the metal center during the reduction process. You see that this is not just like simple 1 or 2 electron reduction this is actually as you see earlier that reduction of 8 electron and which yields 2 molecule of ammonia and hydrogen ok. So, this is an highly reduced state and lots of energy is required and this is why nitrogen is so special.

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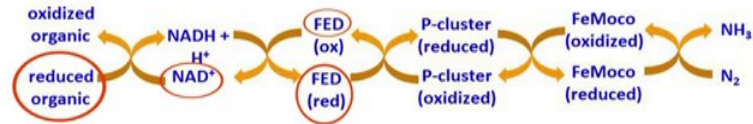


Now, the overall reaction as I have discussed in my previous slide is that nitrogen converting to ammonia and one molecule hydrogen also simultaneously being produced during this transformation with the help of huge amount of energy which is being utilized for this reduction process. So, this process requires large number of electron that here 8 electrons and also huge amount of energy which is provided by the ATP.

So, as one can see that this is iron protein unit which is represented as a green and this ATP binding sites is shown here. So, ATP produces ADP plus phosphate and releases huge amount of energy which is required for this reduction process. And iron sulfur cluster in giving this electron to this P-cluster which actually store all this electron and gives to this MoFe cofactor which is actually responsible for this dinitrogen reduction to NH₃ and produces also H₂ at the same time.

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Nitrogen Reduction Cascade



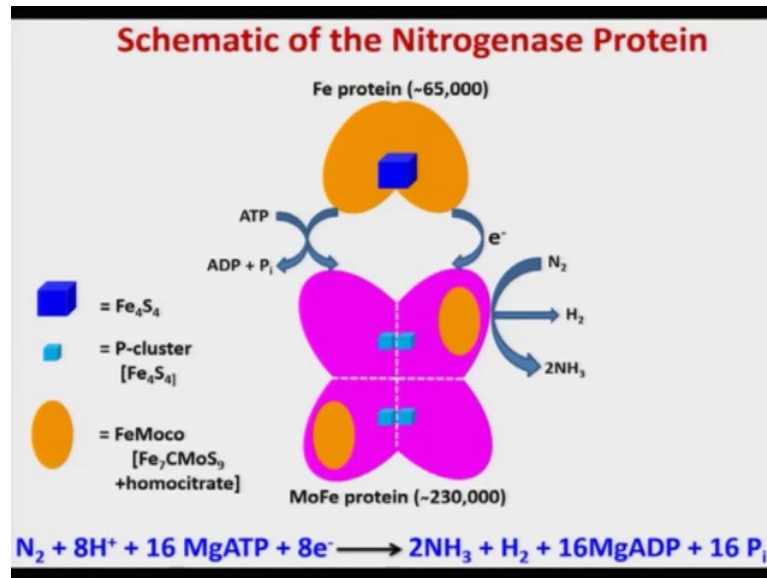
Requires $6e^-$ and 6H^+

Now, how it gets so many electrons which is required for dinitrogen reduction? As you one can see that the reduced organic which gives electron and reduces NAD^+ to NADH and thereby oxidized to oxidized organic. Now, this NADH which is reduced by this way after taking the electron, then gives the electron to ferredoxin and then this ferredoxin getting reduced and at the same time NADH is getting oxidized to NAD^+ which then again start taking one electron from the reduced organic like this.

Now, the ferredoxin which is in the reduced state then gives electron to P-cluster and P-cluster get reduced at the same time ferredoxin get oxidized. So, one get oxidized one get reduced and they are all coupled. So, this is the way electron is transported from reduced organic to P-cluster where it is stored and this P-cluster gives electron to iron molybdenum cofactor and iron molybdenum cofactor get reduced, whereas, P-cluster gets oxidized.

And this iron molybdenum cofactor where actually the reduction of dinitrogen is taking place it requires large number of electron and protons. And this iron molybdenum cofactor gives this electron and this nitrogen is produced to ammonia in this way.

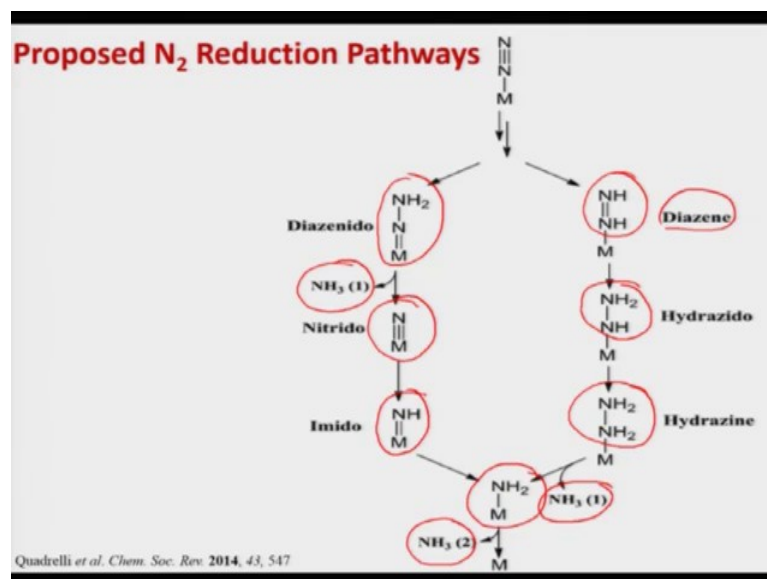
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Now, this is what is the schematic representation of nitrogenase enzyme. As you can see that this iron protein which molecular weight is around 65000 Dalton which is shown in a square block and this supplies electron to the P-cluster which actually indeed store all this electron. And the energy actually 16 ATP unit per dinitrogen is required and this huge energy is provided by the ATP. And, ATP converted to ADP plus phosphate and gives releases huge amount of energy which is required for this reduction process.

And this P-cluster stores this electrons and gives electron to iron molybdenum cofactor and for its reduction of dinitrogen to ammonia. As I have already informed that during this process also hydrogen is being produced. It is not known clearly that how hydrogen is also getting produced during this process, what is the use of that possibly this hydrogen also maintain the very reducing environment which is required for such 6 proton and 6 electron reduction process. So, overall reactions is nitrogen getting converted to ammonia and hydrogen at the cost of huge amount of energy and electrons.

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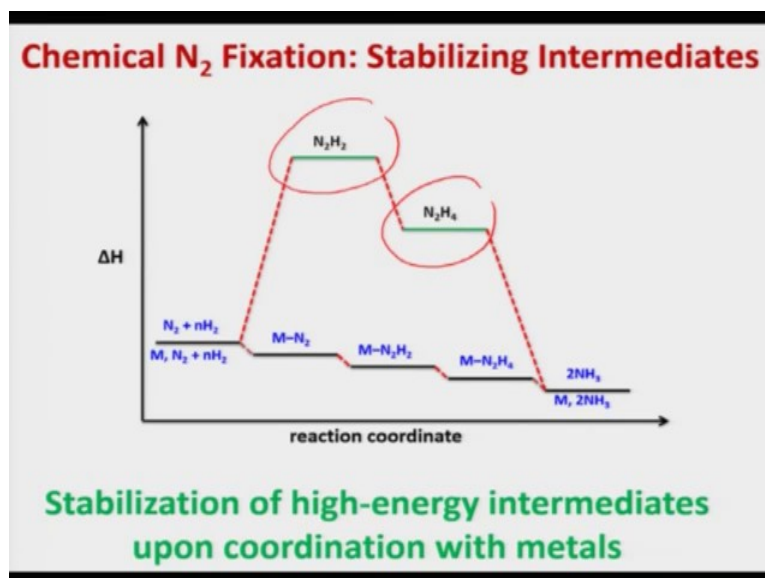


Now, proposed nitrogen reduction pathway. So, it is still not very clear how actually dinitrogen is getting reduced in iron molybdenum cofactor. There are controversies I am not going to enter into that. However, a schematic presentation is shown over here. So, metal binds dinitrogen and this with 2 proton it forms diazene as you can see that this has formed NH-NH and this diazene after addition of one more proton converts to hydrazido NH-NH₂. This hydrazido after giving one more proton it is forming hydrazine and this hydrazine releases 1 molecule of ammonia in presence of 1 proton. And this intermediate which is being formed releases one another molecule of ammonia with the help of one more protons.

So, you see that there are 6 proton 2, 3, 4, 5 and 6, 6 protons is involved to produce 2 molecule of ammonia. There is another way also this reduction is also possible. This first form diazenido intermediate like this where only one terminal nitrogen getting protonated to convert to NH₂ diazenido formation and which then releases one molecule of ammonia with the help of 1 proton and this nitrido intermediate produce imido in presence of one more proton converting N to NH and this imido produce M-NH₂ in presence of one more proton here which eventually releases one more ammonia after addition of one more proton.

As you can see during this reduction process 2 molecule of ammonia is being released and this required 6 proton and 6 electron for just dinitrogen reduction only ok. It is interesting to see that how this metal indeed stabilize those intermediate.

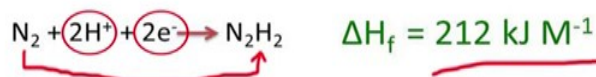
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And this is actually happening as I have shown in my earlier slides that this highly unstable intermediates can be stabilized upon coordination with the metal. So, metal indeed stabilize those high energy intermediate and this is what is happening.

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Chemical N₂ Fixation: Stabilizing Intermediates

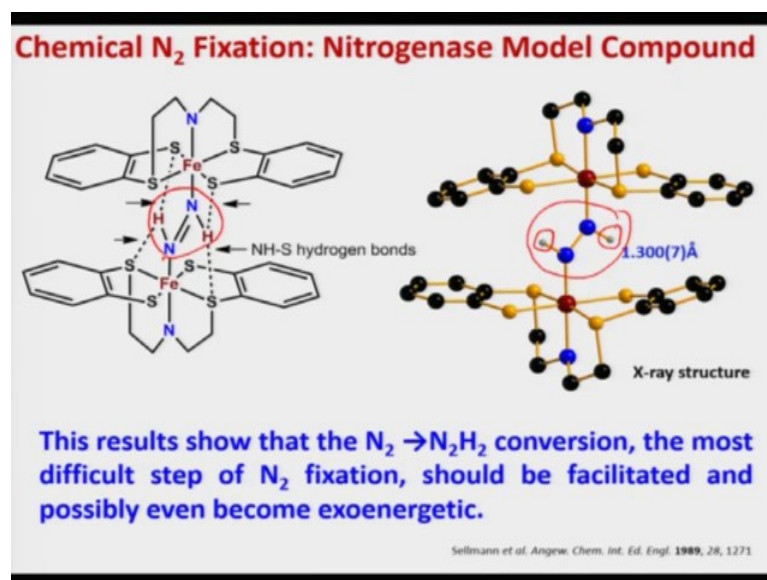


- It is, however, extremely unstable in the free state.
- The question arises by what means it is stabilized in nature at the metal centers of nitrogenase in order to avoid insurmountably high energy barriers in the course of N₂ reduction.

In order to prove this principle people have made various attempt in laboratory and they try to stabilize the intermediates which is otherwise impossible to understand. So, for example, this N_2 converting to N_2H_2 with the help of 2 proton and 2 electron. Now, as I have discussed this is an extremely unstable intermediate in the free state, ΔH_f is 212 kJmol^{-1} in the free state.

Now, can this intermediate be stabilized in presence of metal? Indeed, this is the question arises by what means it is stabilized in nature at the metal center of nitrogenase in order to avoid high energy barrier in the course of nitrogen reduction. This was indeed possible to trap this very unstable intermediate with the help of metal center.

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As you can see this N_2H_2 is being trapped between two iron center here and this is pentadentate ligand which is coordinated to iron and then the sixth position this iron nitrogen bond is there which actually trapped this very unstable N_2H_2 intermediate.

And this intermediate also further stabilized by these NH sulfur hydrogen bonds. The X-ray structure of this molecule was determined by Sellmann *et al.* long back in 1989 and one can see that this N_2H_2 is present over here this N-N distance is 1.300 \AA and this proton has also been located in the X-ray structure which confirms the presence of N_2H_2 between these two iron center.

This results show that this N_2 to N_2H_2 conversion, the most difficult step of nitrogen fixation, should be facilitated and possibly even become exoenergetic. Today, you have seen clearly why the design is so important as the conversion of N_2 to ammonia which is an extremely difficult task can so easily be converted to ammonia in biology by using nitrogenase. If you understand the design principles of nitrogenase correctly, we can then simply implement such knowledge in the laboratory for our well being.

The aerial nitrogen can then be converted to ammonia simply at room temperature and normal atmospheric pressure which would reduce the cost of all nitrogenous fertilizers used by our farmers. Thus food production would be much cheaper than and would eventually lead to green evolution. It would then solve all the food problems in the earth. We are indeed eagerly hoping for that days to come.

Thank you.