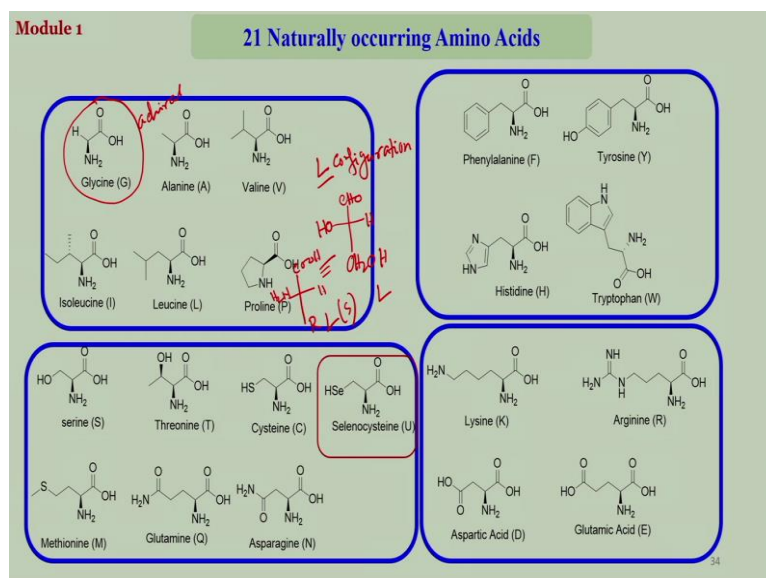


**Essentials of Biomolecules:  
Nucleic Acids, Peptides and Carbohydrates  
Dr. Lal Mohan Kundu  
Department of Chemistry  
Indian Institute of Technology Guwahati**

**Lecture 04  
Amino acids and Proteins**

Hello everybody and welcome back to the biomolecules. So, we have been discussing about the structure and properties of amino acids, peptides and proteins. Most of the biological reactions are as we know are catalyzed by enzymes. So enzymes or proteins are the biomolecules large biological macromolecules which are composed of amino acids.

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And as you know that there are around 21 naturally occurring amino acids in nature and these are the structures. Out of those 21 amino acids one amino acid that is selenocysteine is not a coded amino acid which means only 20 amino acids are coded by genetic code. There is no genetic code present for selenocysteine and it is a non coded amino acid. But Selenocysteine is found in many proteins in many enzymes. But it is not necessarily be present in all organisms not all organisms have Selenocysteine in their protein structures.

So, it is not very common thing if you look at the structure of the amino acids it has lot of various and lot of chemical variations in their structures. For example you start with glycine glycine has one hydrogen here so it is basically a CH<sub>2</sub> here for all other cases and this CH<sub>2</sub> is CH is replaced by some substitutions and all of this amount naturally occurring amino acids have L configurations except of course the glycine.

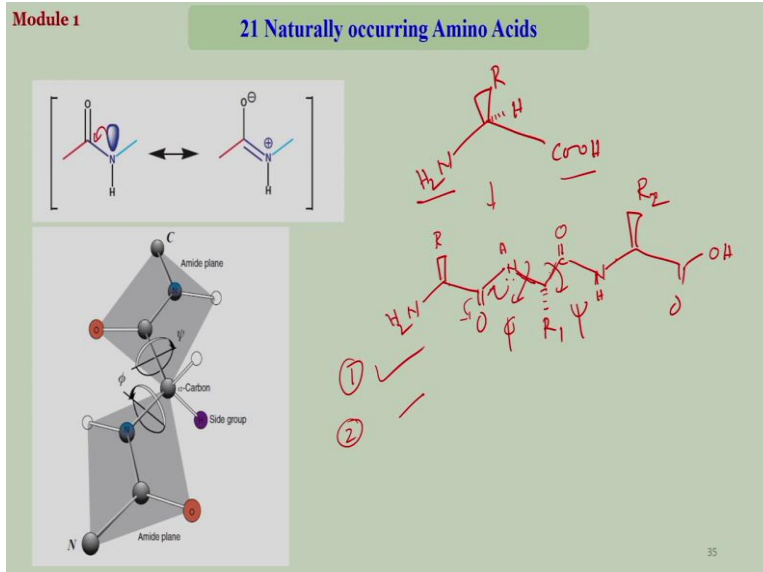
Glycine is a chiral all the other amino acids are chiral amino acids this amino acid is not chiral or a chiral because it does not have a chiral center all other amino acids have a chiral center. And all of them have L configurations natural occurring amino acids have L configurations. And we have mentioned prior that the nomenclature of course came from the glyceraldehydes. If you look this is the structure of glyceraldehyde and this has L configurations sorry there is a OH group.

An OH group is on the left hand side so that is why it is called the levorotatory or L configurations. Similarly if you look at all the structures of the amino acids they have equivalent structure of the glyceraldehyde in this case this is C double OH. I am writing R for general here H and amine here. And NH<sub>2</sub> is on the left hand side so they all have L configurations which ideally means the S configuration. The chemical nature of the amino acids are very different from each other there are a lot of variations as I was saying.

So in this part you can see that this is their alkyl substitutions with branched chains there is a cyclic system in proline which has a second or a minor acid and that makes it very rigid. There are functional groups their hydroxyl groups here which can be functionalized ethyl group here there is a sulphide linkage amide bond here, here. And there are aromatic rings aromatic substitutions with the heterocyclic rings as well. And there are the acids and the basic functional side chains like lysine and arginine have a mean side chains licen has a mean side chain arginine has a guanidine as a side chain.

They are basic side chains aspartic acid glutamic acid have the acid carboxylic acid groups as the side chains.

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So, lot of variations which allows you to chemically modify the amino acids in an enormous way. So, the basic structure of the amino acid if you write the 3-dimensional you can write it in any other way also. I am writing this way where you have the up the plane H down the plane and this is the carboxylic acid end and that is the I mean so this is the general structure of amino acid. And of course the reason it is being called amino acid is because it has an amine group and a carboxylic acid group.

So as group and amine group amine is base carboxylic acid is an acid group and that makes it to have our different structures that I am coming very shortly. If you write a peptide out of it then you will and see not writing the all of it CO and then in H then this will come down the plane R1 R R1 CO NH this will go up again R2 and it will go the end is carboxylic acid and here can be the amine so it goes and the peptide is drawn in that fashion.

You can draw in many other ways and you can see that they have a peptide bond which is basically an amide bond. Now this peptide bond exists in two different structures it can have resonating structures. So, if you look at the peptide structures you see that there are so this bond has a partial double bond character. And apart from this bone other won't like this and this or if you see here this bond and this bond here this bond and this bond here they have pretty much a single bond character.

So ideally if you look the backbone of the peptide or backbone of the protein structures there are plenty of single bonds. So, a protein should have been a very flexible structure because of because this wrote free rotation around a single bond is allowed. And you can ideally you can

rotate the all the single bonds to any directions and to any angles. So, you should have a very flexible moulded structure of a protein. But in reality the structure of protein is not as flexible as you think there are restrictions in there in the orientations.

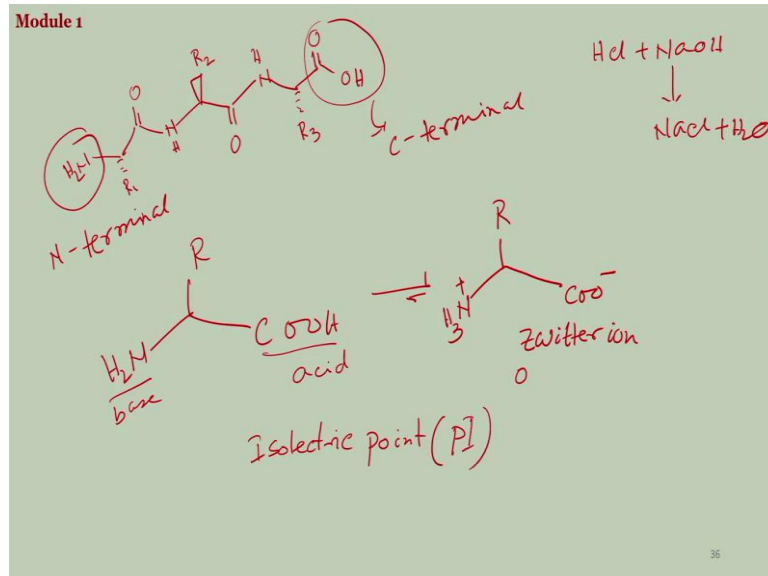
Their basic reason is there are two basic reasons for it number one vision is of course if you look here at the peptide structure then your first reasons is of course the all the single bones that you look here are not actually the single one and there is this peptide one which has the conjugated character as it is given here nitrogen lone pair that makes a resonating structures gives you the double one character here that is reason number one.

Reason number two is there are two planes if you look at the structure here for example if you will see this bond this is the amide bond which is the double bond character this is a single bond. So, this makes one plane which is given here on the other hand this one this makes another plane. And if you look at this plane there is an angle there is a certain angle between these two plane and this is known as the dihedral angle.

The dihedral angle between the two planes, so, if you change the plane ideally you should be able to change the angle because the free rotation around the single bond is possible this is the single bond and this is the single bond and they are named as this is called Phi and this is called Psi. So, you should be able to rotate Phi and Psi too freely. But we will see later on just in a while that if you if you rotate that molecules then the atoms they will sometimes bump into each other and that makes the free rotation around these single bonds not allowed or restricted.

So all rotations around the single bond are not allowed and that was given by a plot called Ramachandran plot that I will just discuss it in a brief while. So, our second thing is that we have seen for DNA that there is a way to write the DNA structure. So, we have seen that you start with the 5 prime end and complete it at the 3 prime end. Similarly for peptide there is a way to write.

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NH<sub>2</sub> CO NH R<sub>3</sub> OH, so you will see that as you move on the right-hand directions you can still continue here when you complete the chain there will be a free carboxylic acid on there on the right hand side if you write in this direction. On the other hand and there will be one free amine group on the left hand side and this is the way to write a protein structure or a peptide structures. That from usually from amine terminal to the and ends up with carboxylic terminal. And this is called the N terminal in terminal of the protein and this is known as the C terminal.

N terminal for amine terminal C terminal for carboxylic acid terminal and this is usually the practice that we use to write us or to represent a structure of a protein. So, before going to the whole protein structures I will come back again to the amino acids and there is a property of amino acids which you already know that I will just briefly discuss. So, I am not writing the stereochemistry now R C O O H I mean this is the structure of an amino acid.

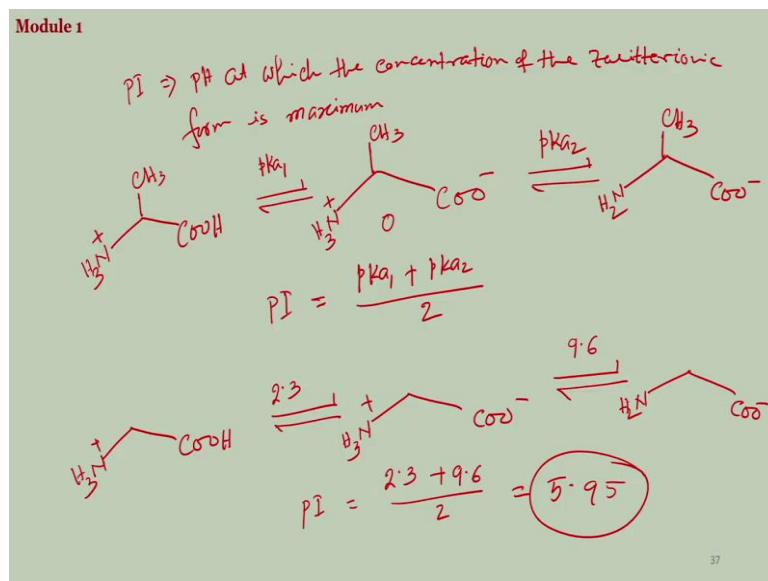
And as I have mentioned that it has a basic group which is the amine functional group it has the acid group and this is acid. So, when you keep a base as well as an acid together mix them together then what do you get for example if you mix HCL and sodium hydroxide then immediately you will get a salt sodium chloride and water. So, HCl if you take that is a strong acid and sodium hydroxide that is a strong base if you mix them together you will get a sodium chloride as the salt plus water.

So acid and base together cannot coexist they will form a salt immediately for amino acids what will happen there are organic acids and organic bases so there will be proton transfer from the asset to the base. So, this molecule does not exist in this fashion it mostly exists in this way NH

3 plus and COO minus. This is what is called the zwitter ionic form, zwitter ion. If you look at the net charge of course the net charge is 0 but it exists in the ionic form.

So, this one does not exist much. The free form of the amino acid exists in very, very less quantity most of the concentration of the amino acids will be here in the zwitter ionic form. And there is one that is where a property called the isoelectric point is coming it is at characteristics not a property is a characteristic of an amino acid that is known as isoelectric point usually represented by PI.

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So, isoelectric point is a kind of an characteristics of an amino acid that is defined as if you have PI is actually defined as the pH at which the concentration of the zwitter ionic form is maximum. So, as you vary the pH of the solution then of course the acid group is there the basic group is there they can be acidity and basicity depending on their acidity and basicity the structure should be changed the proton transfer phenomena would be changed.

So this is the pH at which the zwitter ionic form that is the net charge 0 is the maximum will have the maximum concentrations. Now question is how to calculate the isoelectric point and it varies actually from amino acid to amino acid. so, if you take for example the structure of alanine, alanine has a CH3 here say carboxylic acid. And so how do you calculate the isoelectric point or how old you find out the isoelectric point of an amino acid I am just taking the example of an alanine.

So, usually it is done by a titration you do a titration of the amino acid against a strong base sodium hydroxide usually sodium hydroxide potassium hydroxide can also be used so strong base against the amino acid. Then and you start it with a very low pH and then see how it is going what are the changes that you are having. So, if I now draw the structures of the amino acids starting with the very low pH then what are the structures and that are possible or what are the equilibriums that are possible and then how can you find out the PI from there.

So, if you start with a very low pH acidic pH then all of the functional groups would be the acid will be acid of course the base would be protonated because this is dipped in strong acid. So, the initially what we'll have you will have a  $\text{NH}_3^+$  and  $\text{COOH}$  in very low pH and then as you increase the pH as you titrate it against the in sodium hydroxide you are adding sodium hydroxide slowly then that is raising the pH then after a certain pH what will happen this is an acid which has a low pka value.

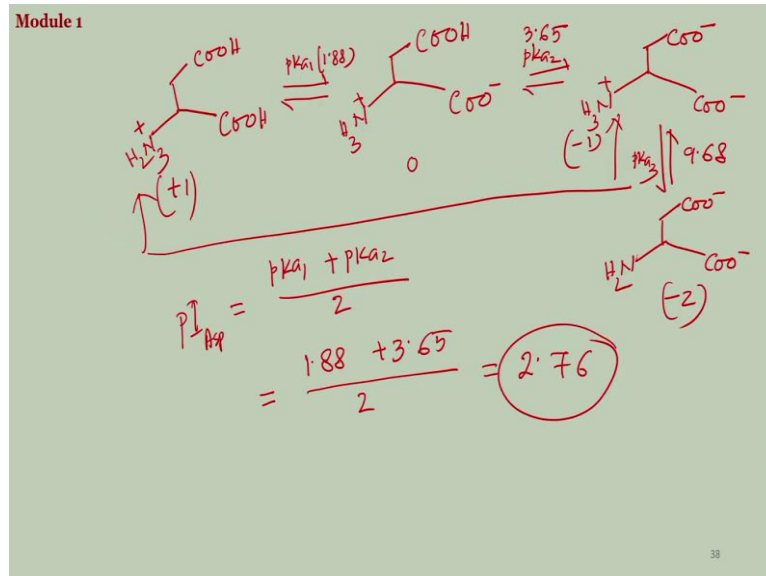
So, that will be deprotonated first you will get this negative charge here this is the acidic group not easily deprotonated. So, this is known as  $\text{pK}_1$  this is the first pk then as you move further on this is minus eventually this should be deprotonated also the  $\text{NH}_3^+$  and will become in  $\text{NH}_2$  so this is your pka minus the second pka so the first pk is for the carboxylic acid and the equilibrium is from the free acid to the carboxylate anion the second pk is for the deprotonation of the  $\text{NH}_3^+$  to  $\text{NH}_2$  the basic.

Now if you look at the values of the pk alanine so your value of PI would be the average of the two because the concentration of zwitter ion around you are basically calculating the concentration of the zwitter ion which is this net charge 0 plus charge minus charge net 0. And this is involved in both the equilibrium this step as well as this step. So, if you want to calculate the concentration of the deuteron you have to take the average of both the equilibrium.

So ideally PI is calculated as  $\text{pK}_1 + \text{pK}_2$  divided by 2 so if I take the example of glycine I have the data for glycine actually here then you can write the same thing the first one  $\text{COO}^-$  minus  $\text{NH}_3^+$  and then this equilibrium still minus and this becomes  $\text{NH}_2$ . Now this pka value is around 2.3 and this pka is around 9.6. so, your PI would be calculated as 2.3 because this is acid so low pka and this is basic show pka should be above 7, 9.6 in this case 9.6 divided by 2 or it is coming as 5.95.

This is the PI of glycine and it is pretty much our characteristics of glycine. Now things are little bit different when you have other functional groups so you have acid and base so if you have a functional the sidechain as another acid or another base then how it would look like how the how would you would calculate the PI.

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So, if we take the example of aspartic acid that is aspartic acid is this there is a  $\text{CH}_2\text{COOH}$  carboxylic acid sidechain. This is acid of course so at very low pH everything would be protonated. So, this would be your first form at very low pH. Now as you increase the pH as you add more sodium hydroxide what will happen your acid would deprotonate but it will go stepwise because the pKa value of this acid and this acid are different.

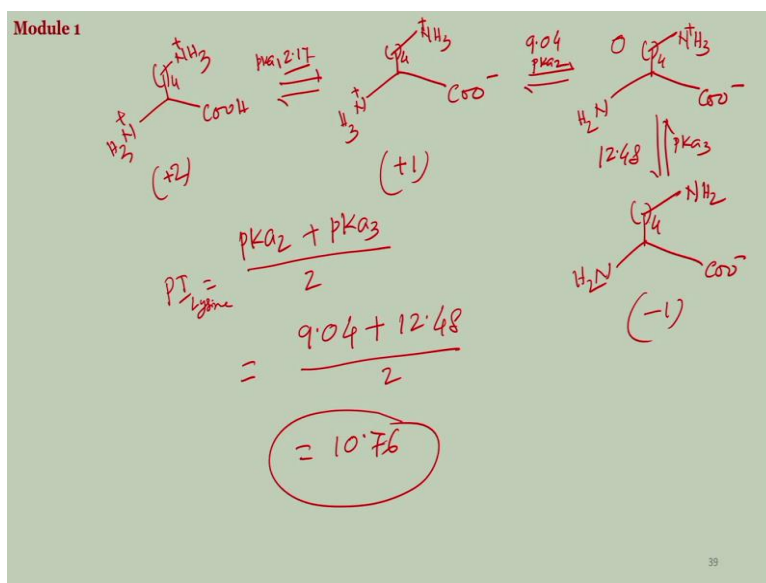
And in fact the pKa of this is low for glycine case we have seen the pKa of this is around 2.3 and you know so this is a derivative of acetic acid kind of thing acetic acid has a pka value of around 4.7 so, obviously this would deprotonated first minus  $\text{NH}_3$  plus. Next is this carboxylate would be deprotonated this acid would be deprotonated minus  $\text{NH}_3$  plus and there would be another equilibrium where everything would be deprotonated.

This so this is pka1, pka2 minus and this is pka3 now the question is which steps you will consider to calculate the PI ideally we are trying to calculate the concentration of the zwitter ionic form. So, where is the zwitter ionic form where the charge is 0 net charge is 0. So, here if you calculate the charges the net charge is here +1 this is where OH is 0 this has a charge of -1 this has our charge of -2. So, if you have to calculate the zwitter ionic form concentrations you have to take these two equilibrium where they are involved pka1 and pka2.



So your PI for aspartic acid would be I am writing a SP aspartic acid would be  $pK_1 + pK_2$  divided by 2 just like the earliest and I have the number only this is one point value is 1088 this value is 3.65 and PK 3 this value is around 9.68. so your  $pK_1$  is 1.88  $pK_2$  is this +3.65 so it is already different than glycine because glycine case and this one was coming into picture divided by 2. So, the average is around 2.76 this is the PI of aspartic acid of course it is way different than glycine glycine has 5.9 in this case aspartic acid has 2.7 then that is because it has a carboxylic acid as the sidechain.

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If you have instead of the acidic side chain if you have a basic side chain then what will happen you have this I am drawing the structure of lysine. Lysine as 4 CH<sub>2</sub> here and then an amine acid NH<sub>2</sub> so again at very low pH everything would be protonated so this would stay as NH<sub>3</sub> plus this should be NH<sub>3</sub> plus as well so the first equilibrium would be deprotonation of the carboxylic acid 4 CH<sub>2</sub> here NH<sub>3</sub> plus this should be minus NH<sub>3</sub> plus.

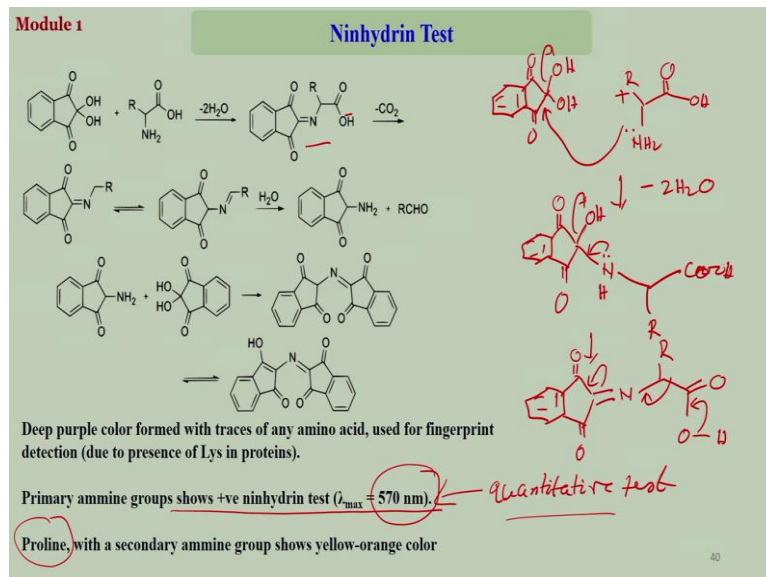
Second one would be deprotonation of either of this amines. So, it turned out that the  $pK_a$  of this amine is less. So, this should be protonated this one would be deprotonated becomes NH<sub>2</sub> and then the sidechain amine this would be deprotonated minus NH<sub>2</sub>. So, this is your  $pK_1$  which has the value of around 2.17  $pK_2$  has the value of 9.04 this is  $pK_2$ ,  $pK_3$  this is around 12.48. Now question is what will be the PI how which steps you will consider for the calculation of the PI.

Again you find try to find out the zwitter ionic form by calculating the charge. So, this has a net charge of plus 1 plus plus this is plus. So, this is +2 +2 here you have +1 2 positive or negative here you have one negative one positive this is your 0 charge and in this case you have -1 so your change of the charge is from +1 to -1 via 0. So, if you want to it is a usual practice that if you want to find out the PI of amino acid.

What you look for is where the charge is changed from a positive to negative via 0 or it can be other way around negative to positive via 0. So, that steps should be considered so your PI would be here is the digital unique form which is so this equilibrium and this equilibrium should be considered. So, it is  $pK_a 2 + pK_a 3$  divided by 2 in this case  $9.04 + 12.48$  divided by 2 that is coming at 10.76. So, this is the PI value of lysine of course it is very again because it has a basic sidechain so the PI value is high. So, that is how you calculate the zwitter ionic structures or the maximum concentration of the zwitter ionic forms.

So, now how do taste up I am I know acid how would you identify and when the presence or how will you taste the presence of an amino acid or a protein.

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So, this is a classic test that is usually been done to find out whether this is a protein or not this is called anine hydrin test and probably you have heard that name and you know this is this is the same test that is used for fingerprint detection in crime thriller or programs you have seen that the people are they are spraying something and then they are fine trying to find out the fingerprint or from there.

They see certain colour usually this is a violet colour or the pink or the purple colour that that appears when you is when you spray that and they actually spray the Ninhydrin solution and that is to figure out whether somebody has a fingertip on it and a fingertip means our even our skin has proteins and amino acids so they if you press it somewhere some of the proteins are very trace amount of the protein would be labelled there.

And it is a very sensitive test to find out whether the amino acids or proteins are present. So, it goes like this this is the solution of Ninhydrin this is called Ninhydrin and here I have shown using the amino acid not the protein. If you have a free amino acids then how the test goes what is the reaction that happens. So, first is if you have the ninhydrin here your OH it is a diol and diol basically and then you have amine group this is R.

So, first reaction is the lone pair I mean is basic and it has a free lone pair of electrons it is a nucleophilic. So, it is a nucleophilic substitution this goes attacks and one OH should be eliminated. And because the carbonyl groups are there they drag the electrons that makes the carbon more electrophilic. So, the nucleophilic substitution of the amine becomes very facile OH you have now NH C O O H and here is your R.

Now once again another one this goes and another way elements so it is minus two molecules of water basically and that gives you this structure a double bond and then you have a carboxylic acid so it gets decarboxylated if you have OH all right. Here this goes this goes it comes back here because again since you have electron-withdrawing carbonyl groups they have the tendency to withdraw electrons that makes this electron deficit.

So it can attract electrons and this carbon can attract electrons. So, you will get this form first with a double bond here and then it gets an originating structures this is more stable. So, it will form this compound. And now presence of water will hydrolyze this bond it is very easy to hydrolyze this bond actually. And if you hydrolyze this if you get ideally our aldehyde and an amine. So, R free amine would be created here which is covalently attached to Ninhydrin.

Now this molecule again reacts another molecule of ninhydrin the same way two times 2 - 2 water so this goes and you get a double bond here. Now this has a lot of resonance it can go here this all the way to the aromatic rings also or it can be the other way around it can stay with

the enolate form. And enolate form will be more aromatic in nature. So, it is because of this structure that gives you the colour.

So when ninhydrin reacts with thymine finally you get this compound and this compound is usually deep purple in color and it is very intense therefore you can use a very trace amount of material very trace even a presence of very trace and quantity of amino acid will show you some colour. This is basically a test for the primary amines apart from the amino acids if you have any other primary amines with a free NH<sub>2</sub> group that will also give you the same taste or the same colour.

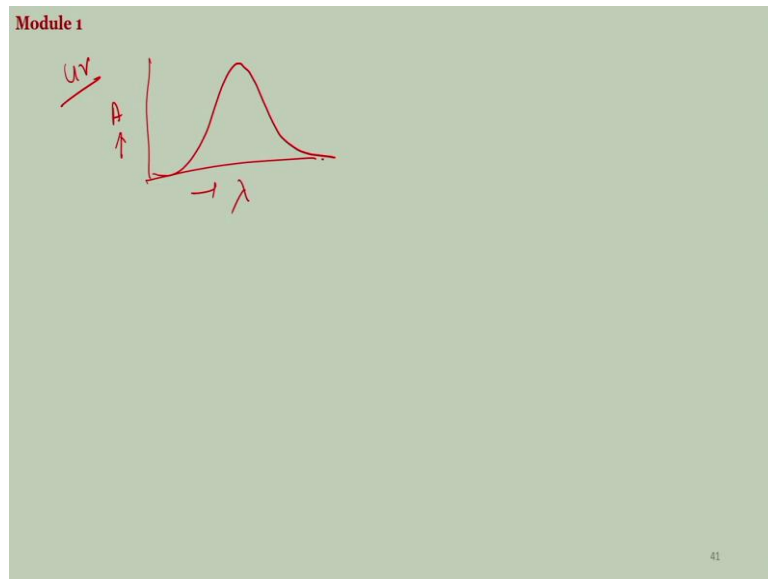
For the amino acids if you have proline, proline has a secondary structure as you have seen secondary amine. So, proline will not give the deep purple colour it produces yellow to orange colour. And now for the normal amino acids if you try to measure number one you can visualize the colour that will give you the qualitative test or it can give you also the quantitative test as well you can determine how much quantity of the amino acids are present there by **by** finding out what is the intensity of the colour.

How much colour you are actually getting and that means if you measure the absorbance of the ninhydrin solution before the start of the test and after the completion of the test then you will see a new peak in the UV region is appearing at around 570 nanometer that depends sometimes on the other water the stuffs that are surrounded to it. Around 570 nanometer wavelength it will show you absorption maximum.

And if you calculate the quantity of it and then you can find out exactly how much of amino acid was present. So, the ninhydrin test can be a qualitative test as well as a quantitative test for the detection of the amino acids. You can use the same test for proteins also because the proteins have free amine as side chains lysine especially and of course arginine is also there both of them have free amine as the side chains.

So, they can react with ninhydrin can give you the same kind of chemistry can give you the same colour. So, this is one test for the detection of proteins second what people do biochemist or biologists do you want to isolate a biomolecule then and you want to know whether it is protein or a DNA or other kinds of biomolecules then you do you can directly measure its UV absorption.

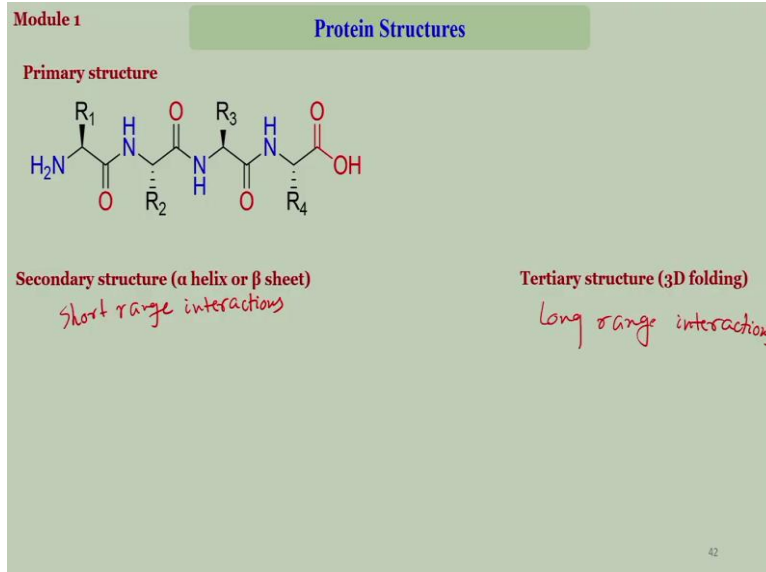
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So, when you look this is the lambda value this is the absorbance UV absorption this is UV visible absorption spectra. If you look at the structure of amino acids you have aromatic compounds which can absorb UV light chromo force basically phenylalanine and tyrosine they absorb a very low region of the UV basically 200 to 220 in that kind of range which is not a very good wavelength to verify.

So, tryptophan essentially that gives you a intense UV absorption sometimes histidine also at around 280 nanometer. So, if you measure you will find if you measure a protein sample the absorption of a protein sample or absorbance of a protein sample you will see that around 280 nanometer you have a lambda Max or the absorption maximum. And this is kind of it is not a really a signature but it is kind of preliminary detection for the proteins once you I as a biomolecule if you isolate from a bio sources. So, these are the two ways that you use usually to for the preliminary identification of proteins.

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Now coming to the structure of the proteins so, I will come to the Ramachandran plot just in a bit time. Protein has this skeleton as we have been discussing again and again with the stereochemistry involved in it. Now this is called the primary structure of the protein which that means simply the sequence of the protein. And then comes how the primary structure of the protein is involved in forming the secondary structure or the tertiary structure of the protein.

So secondary structure means that if if you mold the protein if you since they have single bonds if you place them rotate the single bonds around the protein structures then what are the orientations that you get and what are the short range interactions that are present within the peptide sequence or within the protein sequence. So, secondary structures ideally mean is the short-range interactions and that because of the short-range interactions it has two kinds of mainly two kinds of geometry that is formed.

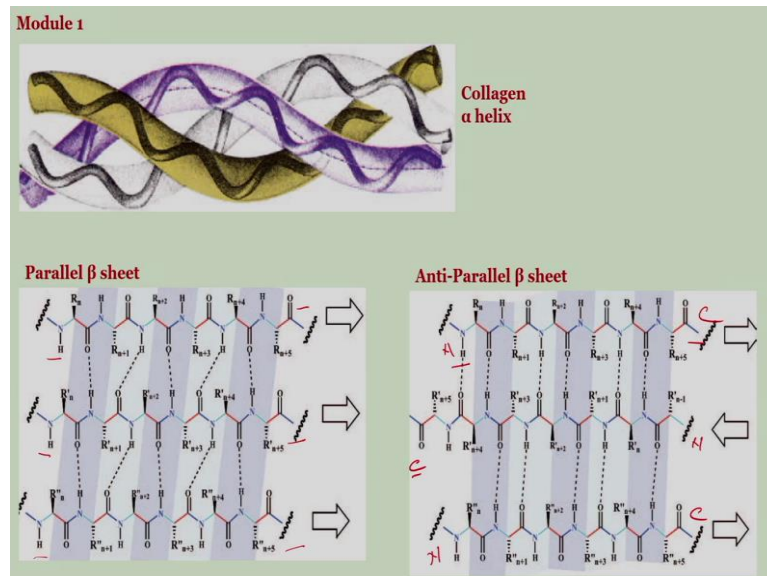
One is called a alpha helix or second one is beta sheet structure. so, these are the side chains. Now if you have for example here I mean as the functional as a sidechain and here if you have acid as the sidechain. So, after certain kind of orientations this I mean free amine and free carboxylate can come in close proximity and they can have electrostatic interaction they can have hydrogen bonding that is just for an example.

So that will change the structure of the total orientation of the protein and that will make the alpha helix or the beta sheet structure. So, this is the short-range interaction - or the local interactions that are present within the protein structure. Tertiary structure is the long-range interactions after you get the secondary structure the protein can mould itself again through our

long-range interactions less like disulfide bonds and all there are other kinds of interactions hydrophobic interactions hydrophilic interactions.

So that a hydrophobic interaction would repel each other hydrophilic interactions will bring them closer so that makes the tertiary structure of the protein or the 3d folding structure of the protein we will discuss one by one.

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So, secondary structure of protein this is how it looks like it can have at the Alpha helix formation or this is a collagen structure which shows alpha helix formations this is a helix that is formed by the arrangement of the primer acids. Or if your orientation is zigzag orientation of the basic skeleton then it cannot form the Alpha helix it cannot turn so it does not form the helix instead they can interact with another molecule of the same protein then again another molecule of the same protein and it will make a sheets a structure that is known as the beta structure.

And again this beta structure can be of two types one is that you have started with N-terminal here this is your C terminal. And the other protein can be on the same direction N terminal and C terminal and then you can see that their backbone this amine and this carboxylic acid they are coming within the hydrogen bond distance these two are coming within the hydrogen bond distance. Like this there can be hydrogen bonds between the two strands of the same protein and in the same orientation in N terminal to C terminal.

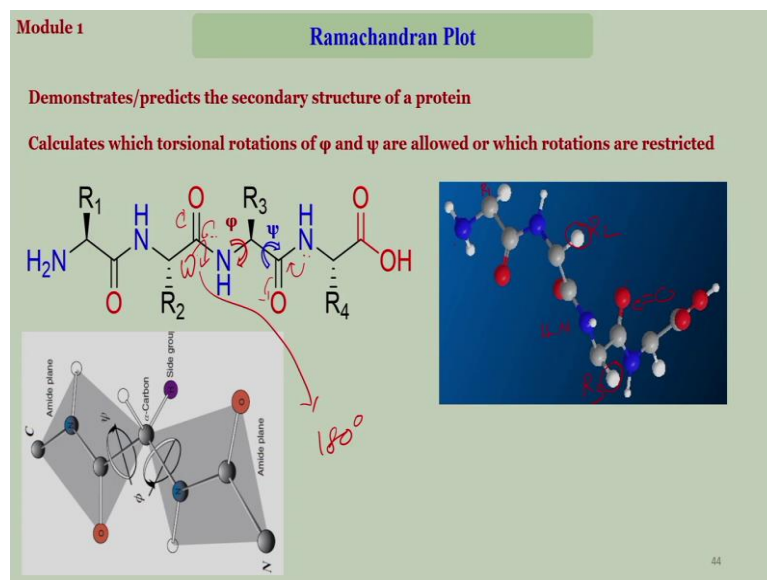
And that can form a hydrogen bonds like this similarly another hydrogen bond can be formed in this way and it will make a flat structure this is known as beta sheet structure. And they are all

parallel we call them parallel because they are all from N terminal to C terminal same directions all proteins all protein molecules. Or it can be the other way around one molecule of the protein is N terminal to C terminal other molecules you just make the other way around.

This is the C terminal and this is the N terminal this is in this is C again this is N and this is C so it goes this way this one is the anti parallel this one is again parallel. So, these two are anti parallel these two are anti parallel and that makes them this kind of orientations one of the amino acid is out cannot form the hydrogen bond the others are forming the hydrogen bond in this fashion this one is also not forming.

This also makes a flat structure so mainly two kinds of secondary structures so these are all secondary structures short-range interactions as I was saying that is due to the orientation of the peptides how much you can flex the peptide or the protein.

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And that is where the Ramachandran plot is coming. Ramachandran plot is a way to demonstrate or predict the secondary structure of a protein which form the protein would assume whether it will be alpha helix structure or whether it will be beta sheet structure that you can actually visualize or that you can predict using the Ramachandran plot. So, what it does it basically calculates two angles the rotation around the two angles one is Phi another is Psi.

So, it is again given here you have one plane you have the other plane the angle between the two plane is called the dihedral angle and this is a single bond this is the single bond as I have told so it is represented here if I take this as a measure then this is a single bond this is a double



bond again this has some double bond character. So, apart from those two the bond is a single bond which is termed as Phi and the other one is termed as Psi.

So it is NHR and it is RCO this these two angles these two are the difference between the two dihedral angles. Now if you move if you fix the Phi and then try to since this is a single bond ideally you can rotate it 360 degree it should be allowed. So, if you rotate this bond then what will happen I have drawn a structure in this fashion here. so, this is amine here and this goes this is R1 this is basically R2 and this is what is your R3 this is this.

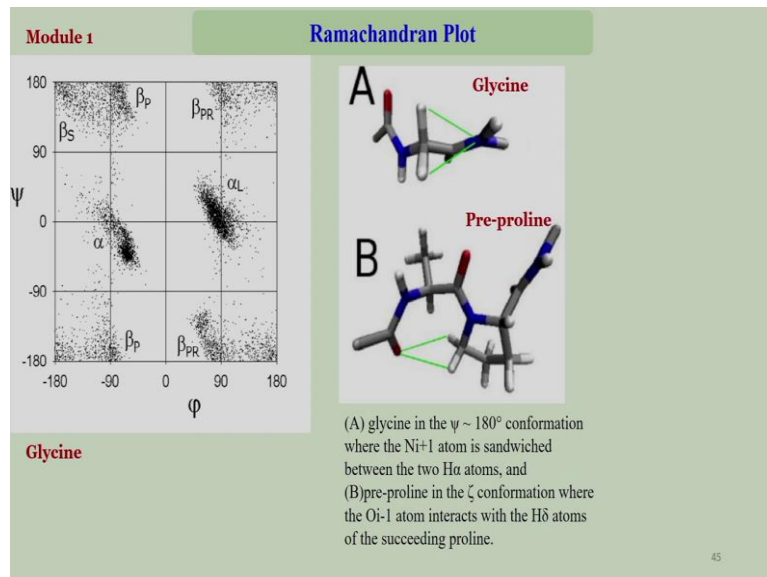
This is NH and this is your CO, so now if you keep this fixed and try to move rotate this bond then what will happen all of what you have on the right hand side would rotate along with it because they are all connected to this. So, once you rotate this bond everything that is present in this direction would be rotating and while changing the rotation you can see here if you rotate this for example 180 degree then this CO will come the opposite direction.

Similarly this R3 will come on the opposite direction and this CO and this NH can come in close proximity. Here you can see this angle is actually known as Omega the peptide angle and of course this is fixed at 180 degree because this is the best to avoid the lone pair lone pair or electron-electron repulsions. So, Omega angle is almost fixed at 180 degree you can rotate Phi and you can rotate Psi.

So you can keep Phi fixed and rotate Psi then you can see that this carbonyl will be upside down will be going up this NH and this CO have 0 angle. So, that will of course change the structure of the protein and that will make also the orientations change of this group and this group very different. In this case they are away from each other if you rotate it for example 180 degree then you will see that this and this will come into close proximity.

And sometimes if these groups are larger then they can bump on to each other and that is not allowed. So, Ramachandran plot actually calculates which rotations are energetically favourable and which rotations are energetically unfavourable and it makes a plot like this.

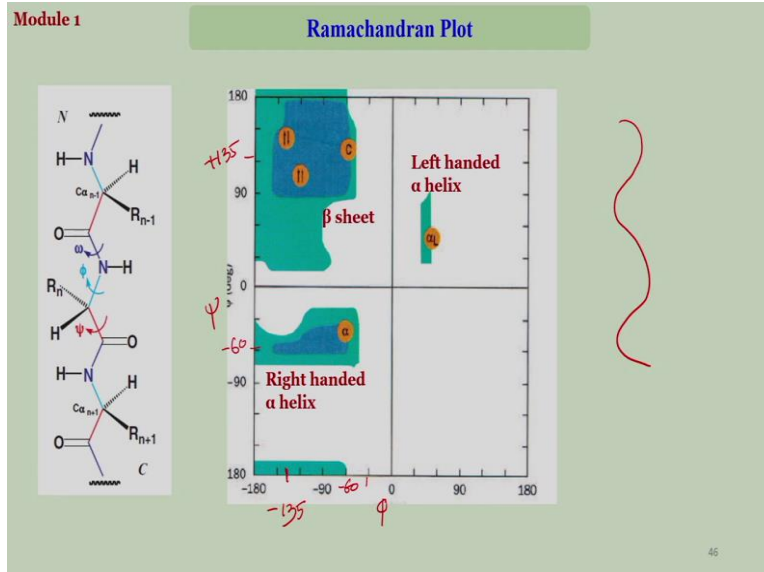
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Since a rotation around of single bond is possible 360 degree the graph is divided into 360 degree minus 180. So, Phi started with minus 180 ends up with 180 that makes total 360. Similarly Phi starts with minus 180 goes up to plus 180 and then you put the points wherever the rotation is allowed energetically allowed. So, if you see that this is the unknown plot for glycine which has h here so the least amount of steric crowding is is involved in glycine and therefore the flexibility should be much more and that is what you see. Almost all rotations are allowed for glycine. See plenty of rotation most of the rotations are fully allowed for glycines and you get all the structures.

If you are if you make a polypeptide out of glycine you can see both beta structures as well as alpha. So, this region is called is known as the Alpha helix this is a right handle of alpha helix alpha L is the left handed alpha helix which is this region. This region is ideal for beta sheets sheet structure so, here it is mentioned. Similarly if you have a proline, proline has a secondary amino acid which has a locked skeleton therefore its flexibility is very less and that will give you a highly restricted structure.

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This is a general structure the general plot for the Ramachandran plot if I take as an example of a peptide the same example I have then you will see. These are the allowed regions all the coloured regions are the allowed regions. So, for example if you have Phi and Psi this is this is Phi and this is Psi. If you have Phi as close to -135 which is somewhere here -135 similarly Phi is also -135, +135 actually here then you will see that they have almost a zigzag orientations.

If you rotate the Phi angle to -135 and shy at +135 you can see our kind of zigzag geometry and since that geometry is good for the beta sheet structures you see here and that is what you see in this case. These are geometry has a beta sheet structure in this orientations this is parallel this is parallel beta sheet this region is anti parallel beta sheet. And similarly if you rotate it to 60 degree -64 for Phi -60 -90 -60 here - 60 - 60 for Phi and -60 for Psi here -60 then it will have mainly the Alpha helix structures.

If you rotate that direction you will see your backbone has to turn here again turns here you get another turn. So, your backbone has to turn in this way and you get the helical conformations will come as right-handed helix and this region will be left-handed helix. So, that how if you change the angle or if you rotate the angles of Psi and if you rotate the angle of Phi then which rotations would be allowed and which rotations would not be allowed is given by our Ramachandran plot.

It is a calculation basically calculation of energy and that finally gives you what will be the secondary structure of a given protein, thank you.