Introduction to Biomolecules
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Lecture – 4
Properties of Water (Part 2/2) and Introduction to Proteins

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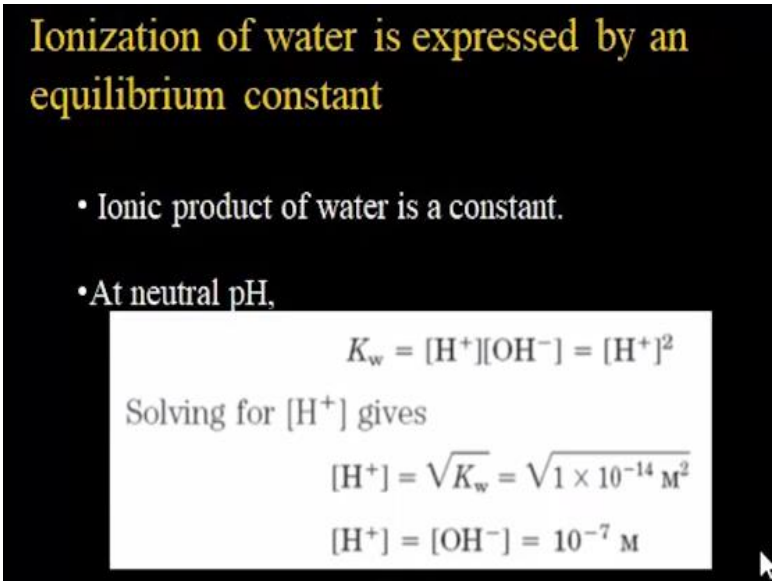

So yesterday towards the end of the lecture we were discussing about water's ionization, ion product of water, pH, etc. So we will continue on that today to weak acids and their behavior and as a consequence of that the buffer system and how buffers are important in biological system. So these are things we will discuss today.

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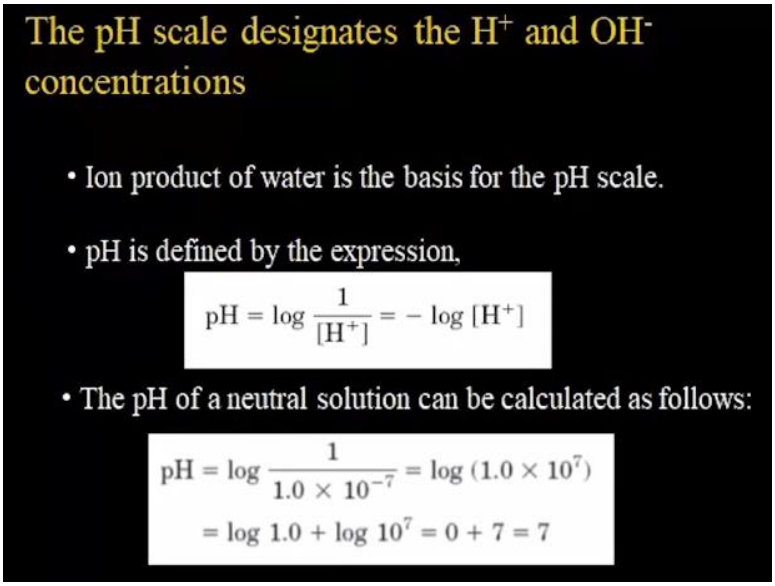

So these are the same slides I showed yesterday.

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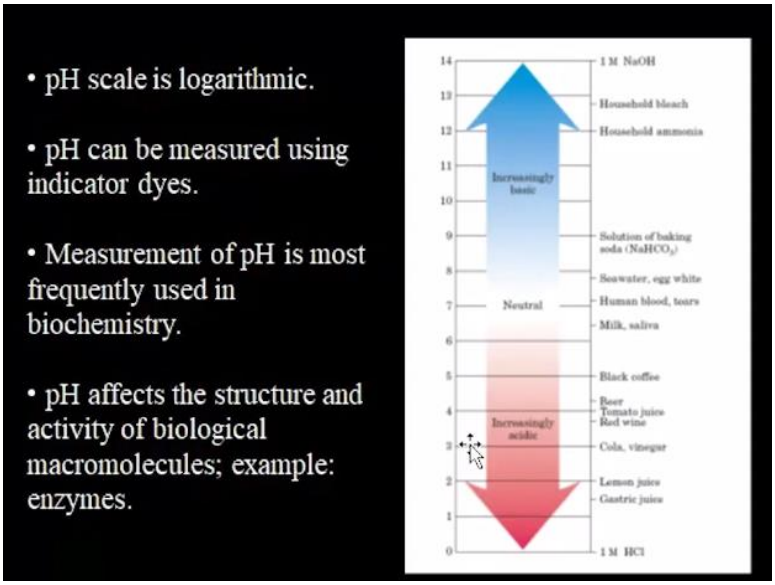

So this cartoon explains the range of pH of the common items that we encounter every day, alright.

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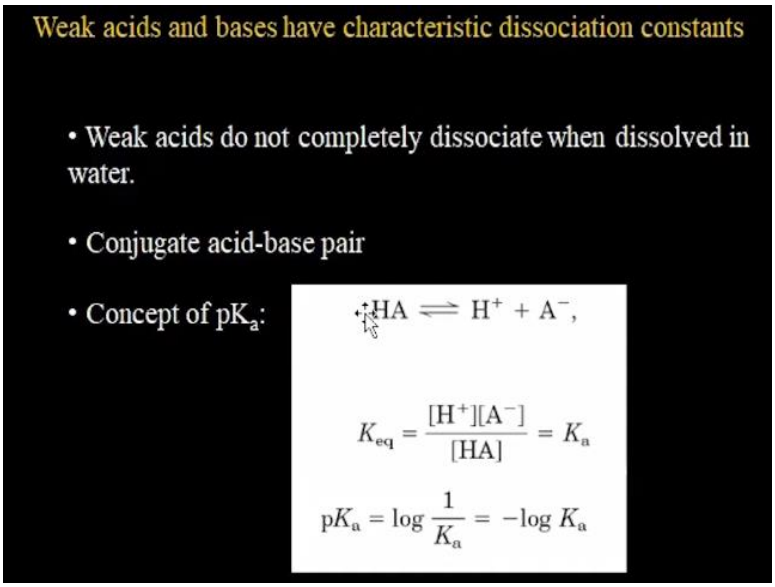

So unlike the strong acids, remember yesterday I was telling a strong acid or a strong base is something that completely dissociates like hydrochloric acid as a strong acid example and sodium hydroxide as a strong base example. So in case of strong acid one molar dissociates into one molar protons, example in HCl, H 2 SO 4 of course one molar H 2 SO 4 will give you one mole of H 2 SO 4 will give two moles of protons and that is where we bring the term normality to describe acids.

So today we are not going to look at the strong acids. In biological systems the most important kind of acids are the weak acids. So weak acids do not dissociate completely, so that is the only primary difference and we are going to look at the consequences of that characteristic. So for example a weak acid here HA dissociates partially to give H+ and A- which can again combine to form HA.

So it is in an equilibrium like A + B giving rise to C + D and the equilibrium constant is C times B concentration divided by A times B concentration, so very similar logic here. So in a product of these two divided by this is the equilibrium constant. And for an acid dissociation we call this as K a, this is the dissociation constant for an acid and a negative log of that just like we took negative log to the base 10 of hydrogen ion to hydrogen ion concentration to talk about pH.

So negative log of this equilibrium constant is a term called pK a which we will often use in discussing weak acids. So the main point to talk about this is this acid which can donate a proton and the resulting base, this A-, this acid and this base, this is base because it can accept a proton in the reverse reaction. So these two, this weak acid and this base that is formed as a result of dissociation of this acid these two together we call as conjugate acid base pair.

So this is a conjugate base for this weak acid on HA. So like for example acetic acid dissociates into protons and acetate ion and that acetate ion is the conjugate base for acetic acid.

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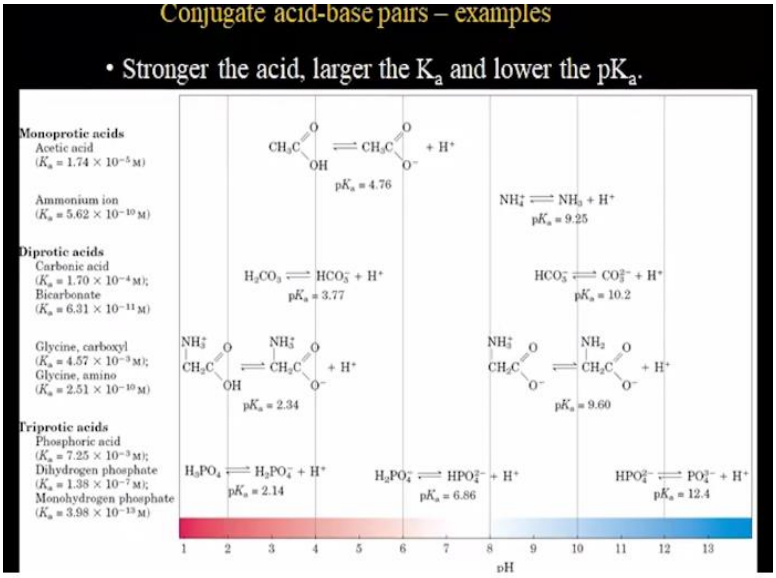

So here are some examples of weak acids and with their pK a values given. So here you have acetic acid, its pK a value is 4.76. What it means we go back and look at this when our concentration of the numerators and the denominator are equal, so then that will be the point that pK a indicates. So in this case 4.76 is the pH at which these two will be in equal concentrations like the dissociation will be half of the acid and similarly this combining will be half of the concentration of this.

So that a half way point whatever be the pH at which these two will reach halfway will be the pK a, so that will become clear when we get to an equation that talks about this feature. So at 4.76 pH, 50% of this would have dissociated that is what it means. While this one to dissociate 50% the pH will have to be 9.25. So this does not readily yield protons, so this is a base, so that is clearly indicated by this pK a value.

Now you look at the other one like carbonic acid, its pK a value is 3.77. That means even in the lower pH than the one for acetic acid 50% of this will dissociate. Meaning this is a stronger acid than acetic acid. And you look at the glycine which is an amino acid, you know NH 2 amino group and then the carboxyl group. So its R group is another hydrogen, therefore this is written as CH 2. So this one here the carboxyl group dissociates at 2.34.

So you pay attention to this, this is carboxylic acid group which is the same as the carboxylic acid group in acetic acid, but you see this will dissociate 50% at a much higher pH than this. So the carboxylic acid in glycine is more acidic than the one in acetic acid. So you look at the

difference between the pK a. So again pK a represents the pH at which half of the molecules the acid would have dissociated.

So lower the pK a value that means stronger the strength of the given acid. So this difference is primarily, I asked you to do this as a homework you read it for yourself; I guess I leave it at that. I am sure some of you will be curious and read and figure it out that way you will remember it better than me right away giving the answer. And this one interestingly has another group that can be protonated or that can donate a proton depending on the pH.

And that will dissociate at a much higher pH because it is a base group so at 9.6 a half of it will get dissociated. Meaning in the surrounding medium, the proton concentration has to be so low for it to dissociate. So these are the diprotic acids, you have two groups that can be protonated or can dissociate. So, triprotic acid the most common example that we encounter in biology is the phosphoric acid.

We have already seen it in the context of phosphodiester bond in the nucleic acids and we also saw it in phospholipids. So this has 3 acid groups, you know H 3 PO 4. So, each one of them have different characteristic pK a values. So the first one dissociates at a very low pH indicating it is a stronger acid group and then next one at a higher pH and the other one is really at very high pH, it is nearly behaving like a base.

So, this H 3 PO 4 and H 2 PO 4 can act as conjugate acid base pairs and this H 2 PO 4 and then this HPO 4 can act as conjugate acid base pairs. So, this will be acid, this will be base and for this this will be a conjugate base and so on.

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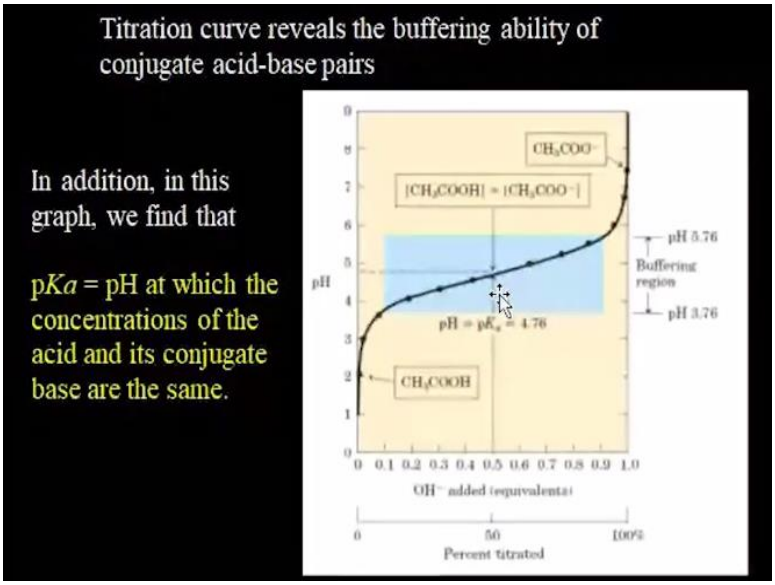

So an important aspect of weak acids is the titration curve shape. So here all that we are doing is we are titrating acetic acid. So you are taking concentrated acetic acid and then you are adding hydroxyl ions, let us say we are adding sodium hydroxide which will readily dissociate and give hydroxyl ions. Now that will make the pH to increase and as the pH reaches a point where this carboxylic acid group will dissociate it will start dissociating.

You see as you add the hydroxyl group the pH increases and after increases to a certain level it does not increase that rapidly, the slope starts to decrease and then you reach a point where half of the acid is dissociated and therefore the concentration of the acid and the conjugate base are same. At that point the pH is equivalent to the pK a 4.76. So at that midpoint where these two are equal, the pH reaches the pK a for that acid.

And the main point we really need to pay attention to is this shape, this range like marked here 3.76 to 5.76**,** in that range for the quantity of hydroxyl ions added or the base added the pH does not proportionally increase. It sort of resists a change in pH in this range. So, revealing that the dissociation of weak acids can actually reach a buffering, it sort of brings out a buffering ability of these systems, this conjugate acid base pair resists change in pH when the pH is near the pK a value.

So, on both sides you have some leeway but this is the midpoint where the change is really, really the lowest. So this is the basis for the concept of buffer. So, buffer is a solution that resists change in pH when you add a certain amount of acid or base. It is not going to tolerate

forever, if you add a lot of base then it is going to dissociate and then the pH is going to start to increase again.

And similarly, in the opposite direction again if you add acid it is not going to resist continuously forever, once you go beyond a certain level then the pH is going to start to drop. So in a narrow range depending on the molar concentration of the buffering system that you have it will resist the change of pH to a certain concentration of addition of acid or base.

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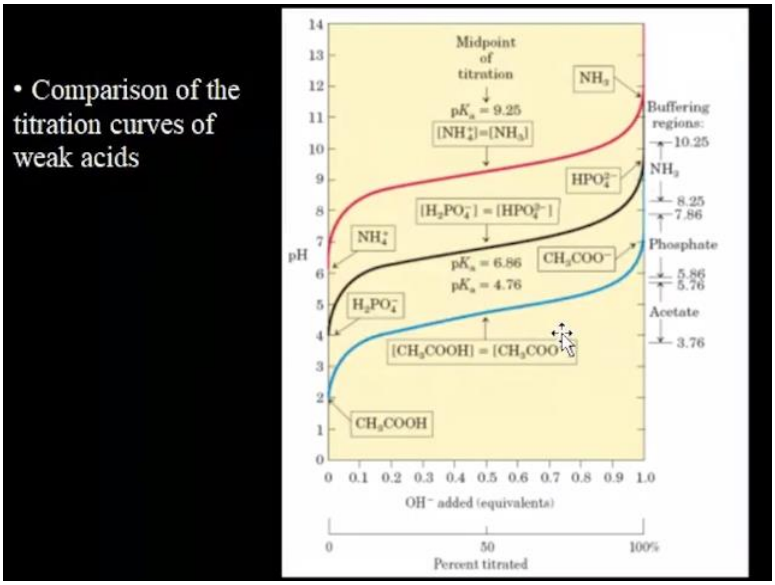

So, this is a same thing titration curve for different weak acids. So they all have the same shape, but the difference is they are kind of upshifted if you are going from acetic acid up towards this ammonium solution or if you go in the opposite direction then it decreases. So in this graph among these three examples, acetic acid is the strongest acid which dissociates at a lower pH.

But the point is all of them have the same shape indicating a fundamental relationship, a fundamental principle that govern the dissociation of weak acids and that is essentially the two main points that is when the pH reaches a point where the concentration of the acid and base are equal that is the pK a value and second around that range these systems resist change in pH when acid or base are added.

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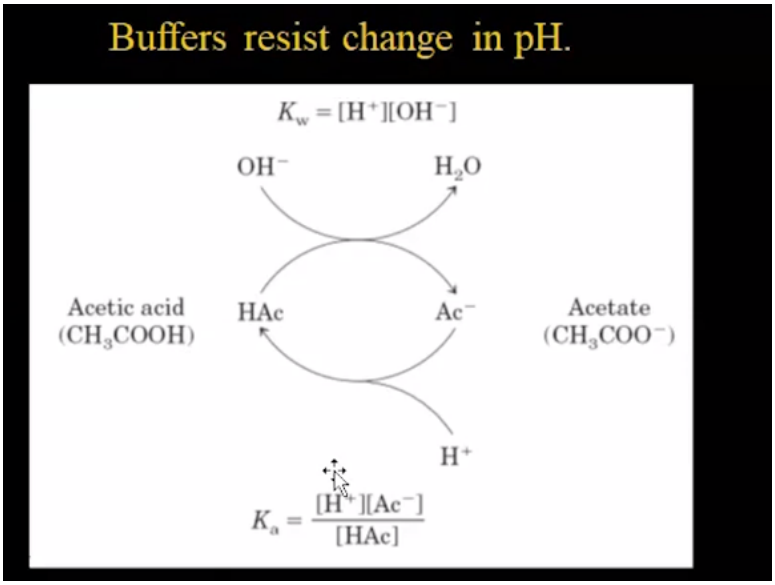

So this is the coming specifically to the buffer. So buffers resist or change in pH. So how does that happen when you have an acid when you add base to it then the proton is taken up by this converting into water and therefore the pH does not change because the proton has been taken out. And similarly, this is the proton and here when you add the negative charge the hydroxyl group it is taken out.

So in this case proton is taken out by the conjugate base making it into acid. So these two reversible equilibrium; with characteristic equilibrium constant is the reason why these systems behave as buffers. If they were not having this ability to partially dissociate, this partial dissociation is the principal reason why we are able to reach a situation where the reversible situation becomes possible. In the case of strong acid where they completely dissociate, this is not going to happen.

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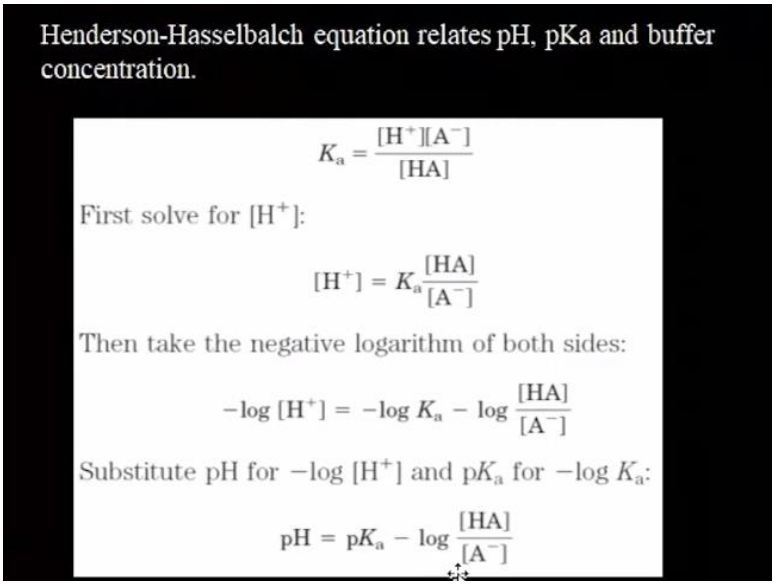

So to rearrange this equilibrium constant in a way that becomes practically useful to calculate the different entities like for example how to determine the pK a value or how to find the pH if I know the pK a value and if I know these two then what is the ratio of the acid and the conjugate base, so for those calculations it really becomes useful when it is rearranged into this form and this is called the Henderson-Hasselbalch equation.

This relates the pH, pK a and the ratio of the conjugate acid and base. So this is quite simple, you start with this equilibrium constant that we are all familiar with. Then you rearrange it for H+, then you take negative log on both sides, then you change the sign then you get this equation pH = pK a less negative log of H+ is pH and negative log Ka is pK a.

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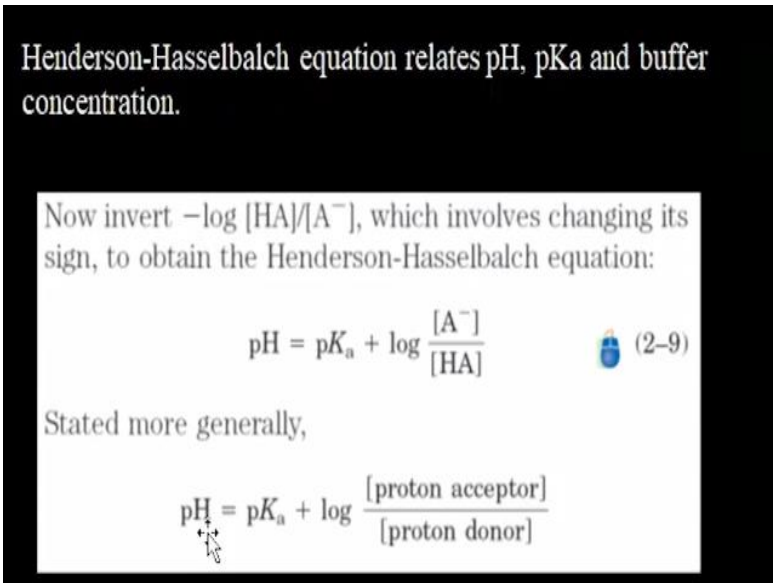

And so this you can rearrange to make it A divided by HA so that is what is here, so it becomes plus changing the sign here okay. So proton acceptor divided by donor, so this equation is very useful like for example if you know these ratios you can readily calculate pH since this is known for all weak acids already. And similarly if you know these two, you can calculate these ratios and this helps us to make buffers.

In what ratio should I mix the base and acid to get a certain pH and also given the type of reaction you want to do what is the pH range in which you want buffer action and if you know the pH range in which you want a buffer then if you know the pK a value of many different conjugate acid base pair and you can choose the right one where the pK a will be near the range you want. For example if I do a reaction let us keep this.

So I am going to do an experiment where I want the pH to be somewhere around 5. Then I know pK a for acetic acid is like 4.76 close to 5 and therefore this will be a good buffer around the range I want. So I will take this as the buffer but not this one because this is not going to behave because it will already be fully dissociated and the pH will readily increase, so it is not going to act as a buffer at the pH that I want.

So knowing all of this helps us to make the right buffer, right concentration all of that. So once you determine the range in which you want and select the acid and even within that what ratio do I need to take to get the right pH of the buffer.

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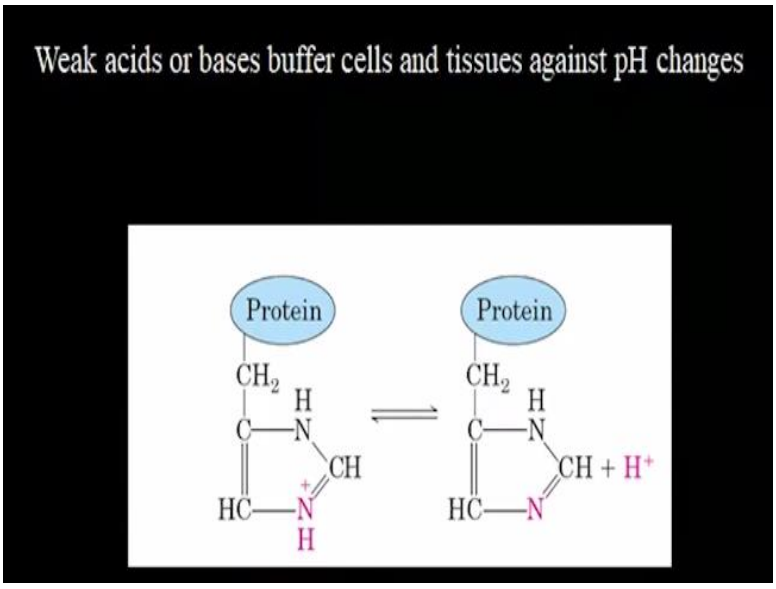

So why do we need to worry about buffer so much? You know our goal is to understand the chemistry of living systems. The primary reason is the living system deals with a lot of protonation and proton donation and those things are critical for reactions and as a result the hydrogen ion concentration becomes very crucial. And if the hydrogen ion concentration is crucial, then maintaining the hydrogen ion concentration in a certain range meaning a buffering action is also important.

So a lot of constituents of our polymers themselves can act as these conjugate acid base pairs. For example, you look at the side chain of an amino acid. So this is a histidine, this is imidazole ring, so here you have you know a group that can donate proton and it can accept proton. So this forms a conjugate acid base pair. So like this there are many other groups. There are other amino acids as well that can do this.

And individual amino acids have two charged groups, we already saw it in the glycine you know, the amino group can be protonated to NH 4 and carboxylic acid group can dissociate proton, so you have two groups already in every amino acid, but of course they are going to participate in the polymer like in the peptide bond but the side chains like this imidazole group and the amino group in the side chain of lysine.

And you have aspartic acid, glutamic acid having carboxylic acids in their side chain. So the guanidino group in arginine all of these are charged molecules that can act as weak acids and bases that is one.

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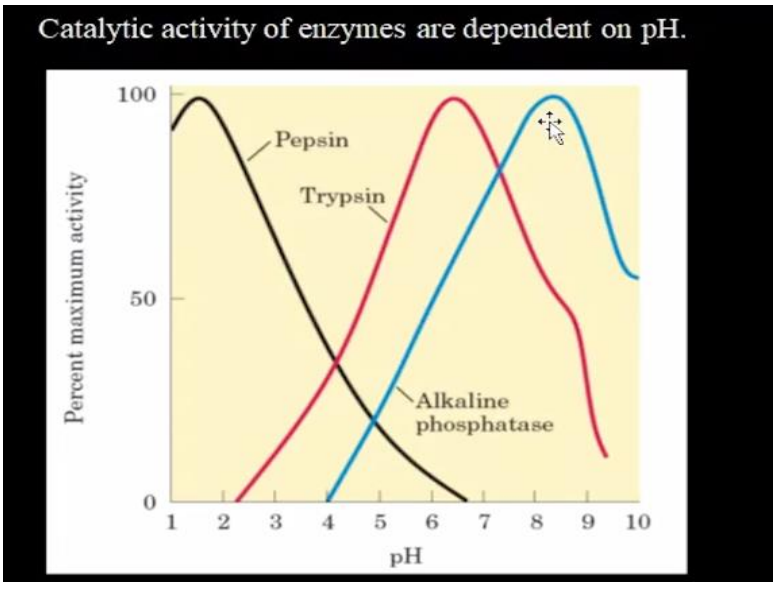

And second our enzymes each one has a characteristic optimum pH at which their activity is maximum. So that is shown in this graph. So if you take pepsin, so the pepsin is an important enzyme present in our digestive juice that digests the proteins that we eat, the protein components of our food gets digested by pepsin that works at pH 1.5 that is where its activity is maximum at neutral pH basically no activity at all.

On the other hand, if you take the intestinal alkaline phosphatase which removes phosphate groups from variety of molecules that works around 8.8 or 9 pH that is where its activity is maximum and at 1.5 it is not going to be active, like at 4 it is going to be 0. On the other hand, trypsin that is another protease on that box near at about the neutral pH. So each enzyme has its own characteristic pH and therefore the hydrogen ion concentration becomes very important.

So when you purify these enzymes and use in molecular cloning or other biotechnology applications in the lab, you will make the buffer that is the right one for a given enzyme that you are working on.

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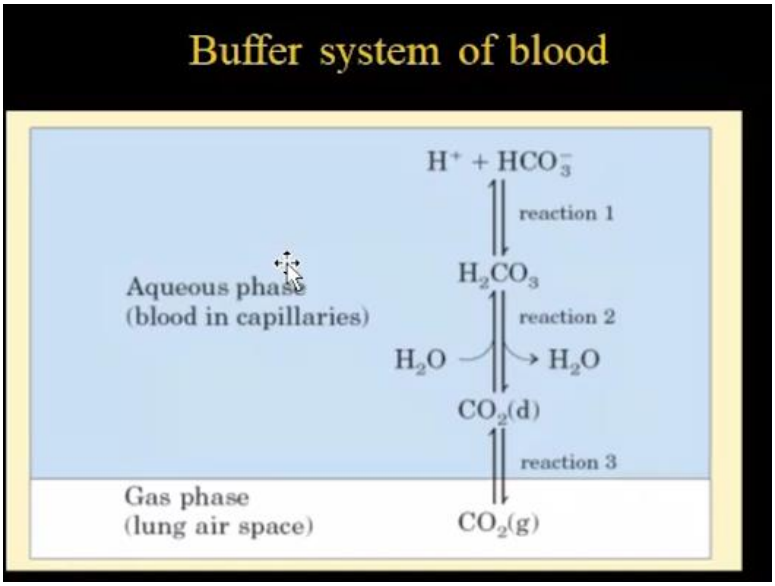

So the previous graph tells you the importance of the pH for the enzymes and the enzymes being crucial the importance of pH for the life as a whole, but in the one before I told you how there are charged groups in our biomolecules that can act as weak acids and bases and therefore some buffering ability in them. But they are not enough to handle the large volume of proton formation and the consumption in our whole system.

And for that there is a really sophisticated buffer system that exists in our blood and that is the carbonic acid, H 2 CO 3. So this one can dissociate forming this bicarbonate ion and proton and it can also dissociate into CO 2 and water. So essentially when carbon dioxide dissolves in water, you form carbonic acid. So the concentrate that the beauty of this buffer, okay before I explain and makes you appreciate its sophistication let us look at a normal buffer.

So let us take for example, let us take acetic acid. So if I have a certain quantity of acetic acid in the solution, let us say I have 1 molar acetic acid 100 ml. Now it will start to dissociate as I add sodium hydroxide when it is completely dissociated then that is the end of it. When it is half way dissociated and if I continue to add sodium hydroxide, then the pH will start to climb.

So it cannot resist the pH change anymore and there is no way in the middle of this reaction I can vary this concentration of this concentration, they are fixed. Whatever I started with that is what is going to be there in the solution. So if I want a higher buffer capacity that is if I want more sodium hydroxide addition to be tolerated without pH change, then I should start with a higher concentration of acetic acid.

So that is how you decide whether you want 10 millimolar buffer, 100 millimolar buffer, 1 molar buffer and so on. So, that the concentration of the buffering molecule determines how much acid or base can be tolerated. So if you want to increase the quantity of the additional acid or base to be tolerated, then you have to increase the concentration of the buffer. So that is automatically possible in the case of carbonic acid.

Simply because our lungs can take in required carbon dioxide to make more carbonic acid or they can throw away carbon dioxide to reduce the carbonic acid concentration. So this concentration can be dynamic and as a result this can have a flexible ability in terms of its buffer capacity. Buffer capacity can be increased or decreased, it is not fixed and that is purely because of this reaction 2, reaction 3 equilibrium here.

So that is why this carbonic acid forms the main buffer of our blood. So carbonic acid concentration in the blood can be readily varied by taking in enough of this gaseous carbon dioxide into the dissolved form or dissolved the carbon dioxide into gaseous form. So you

can tweak the concentration of the carbonic acid. So that is the uniqueness of the buffering system in our blood and this is what helps us to really act as the main buffer of our physiology.

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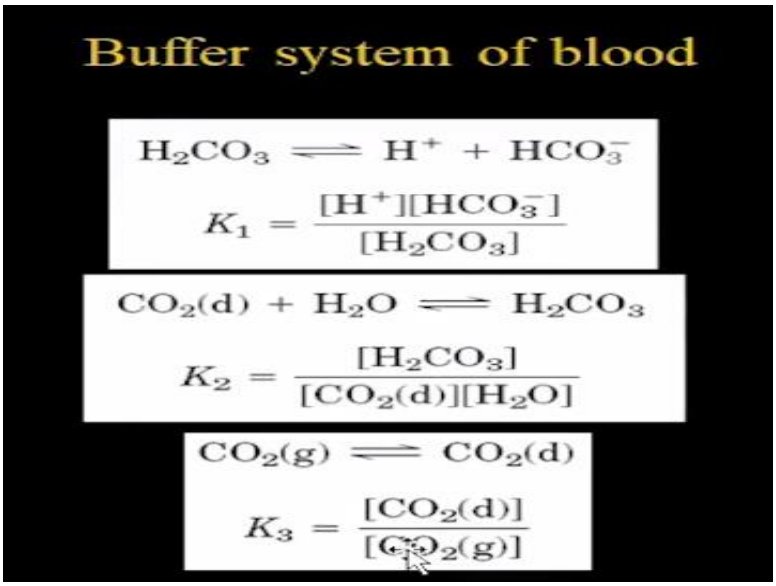

So these are the equilibriums that are easy for you to understand. You have a reaction 1, 2 equilibrium here and then here an equilibrium. So you kind have our 2, 4, 6 rate of reaction equations and 3 equilibrium constant equations and that is what is shown here K1, K2, K3 and so all of this put together really makes that buffer system very sophisticated, each one can be tweaked.

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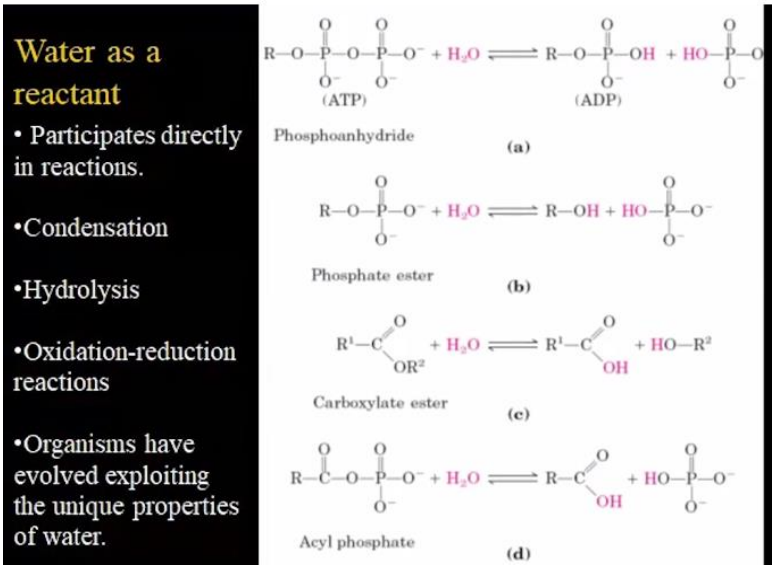

So still although we are talking about proton donation, proton acceptor, conjugate acid base we are actually appreciating the beauty of the water molecule, in all of these it is the water, so

water is where this acetic acid is dissolved or diluted our carbonic acid is present, etc. So it is all still connected to our conversation on water that we started two lectures ago. So in addition to all these uniqueness that comes from the electronegativity of that oxygen atom in the water molecule.

Actually participates in the reactions themselves. It is not just an enabling environment or enabling medium, this is also a reactant okay, a substrate for an enzyme and so on. So those examples are given here. So here you have anhydride bond between two acid groups getting hydrolyzed, so this is hydrolysis where water is a reactant and condensation reactions where the reverse would be condensation ATP formation, inorganic phosphate and ADP condensed to form this releasing water.

And oxidation reduction reactions, there again water molecules participate. So in this manner, water itself is one of the reactants in biological systems. So organisms over the course of evolution have exploited the uniqueness of water to adapt to environments. There are many adaptations that we find, like for example you sweat and the evaporative cooling cools your body and helps you to maintain a constant body temperature and that is why people say in hot weather sweating is good.

So if it is hot, but not humid you actually can have heat stroke. If it is hot and humid where you sweat and do evaporative cooling you do not dehydrate readily and your body maintains temperature. And similarly, transpiration of water from a plant leaves helps them to control their processes and when the surface of the water bodies freeze because they are exposed to cold air atmosphere the ice that floats on the surface.

Because ice has a certain fixed orientation of the water molecules due to that the density of the water molecules are not tightly packed. As a result they are less dense than the liquid water and the ice floats on the surface and that acts as an insulation and prevents water below the surface and that protects the living beings at the depth of the water bodies. So like this in many different ways organisms have adapted on to these unique features of water.

So if you are going to find the organisms in a foreign planet where the solvent is not water and those organisms may have nothing to do with us at all. So they may not have any resemblance to us. On the other hand if you are going to find organisms which resemble the

ones on earth that means that environment is likely to have water okay, so water is everything, right.

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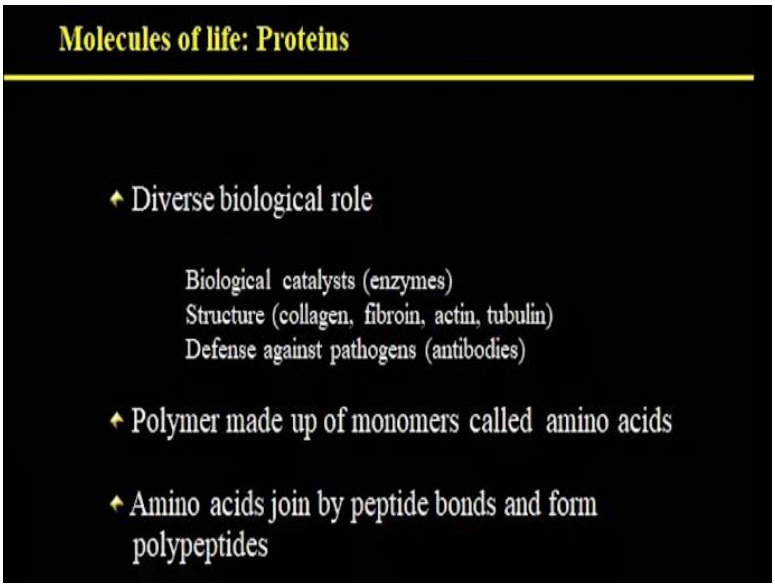

So now what we are going to do is we are going to move from water to the next major macromolecule that we have already seen, but now we are going to get into details of it and that is proteins. So the reason is from proteins we are going to move to enzymes and then we are going to learn about how bio catalysis and thermodynamics, etc., work and that is the reason we are beginning with proteins and then we will go to carbohydrates later.

So our goal is slowly move into enzymes because enzymes means then we will directly be talking about biochemical reactions and when we are going to talk about biochemical reactions there it self we can discuss the energy transformations in the biological systems because that happens via biochemical reactions, and therefore we can try to think about and understand how the laws of thermodynamics are obeyed by the biological systems.

And also we can learn about the kinetics of biochemical reactions and that will set the stage to discuss metabolism. So let us begin with proteins. So all of this will look complicated, but if we go one topic at a time these are all easily understandable intuitive concepts so you will not face any great difficulty in understanding these concepts, so you will go one at a time. So we already know the proteins are catalysts and in addition they have structural role, defense role all that we saw.

They have diverse biological functions and they are polymers made up of amino acids and amino acids are joined by peptide bonds, right.

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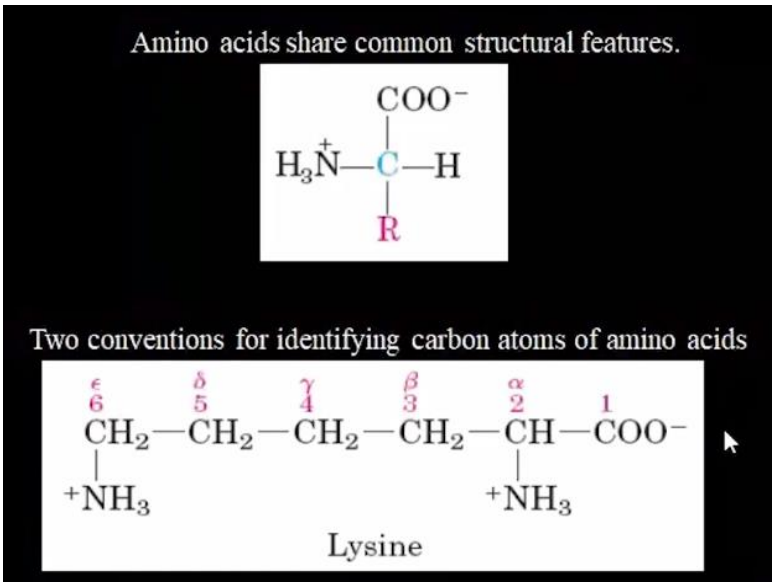

So now let us look at an amino acid closely, so shown here is a generic amino acid where the side chain is denoted by this letter R and so this is one specific amino acid lysine, I mentioned earlier that it has a charged group in its side chain. So this R group is what I keep referring as side chain and so this carbon to which these are all added we saw is a chiral carbon except in the case of glycine where the R group is another hydrogen and this is how the numbering works.

The carboxyl group is number 1 just like in any aliphatic acids, carboxyl group the functional group is where we start, but by convention people started earlier this central in terms of the moiety is attached to it as alpha carbon. So therefore we have two concurrent naming schemes here. So you have 1, 2, 3, 4, 5, 6 where 1 is the carboxyl carbon and an older convention that is still continuously used like for example structural biologists will always refer this as alpha carbon.

They will say C alpha bond and NC alpha bond. So when they say this alpha is this carbon. So going by that this is alpha, beta, gamma, delta, epsilon. So often this is called epsilon amino group, nobody is going to say sixth carbon amino group, the most of the people will talk it as epsilon amino group of lysine. So therefore try to remember this and its relationship to the most standard nomenclature which is this carboxyl group being 1.

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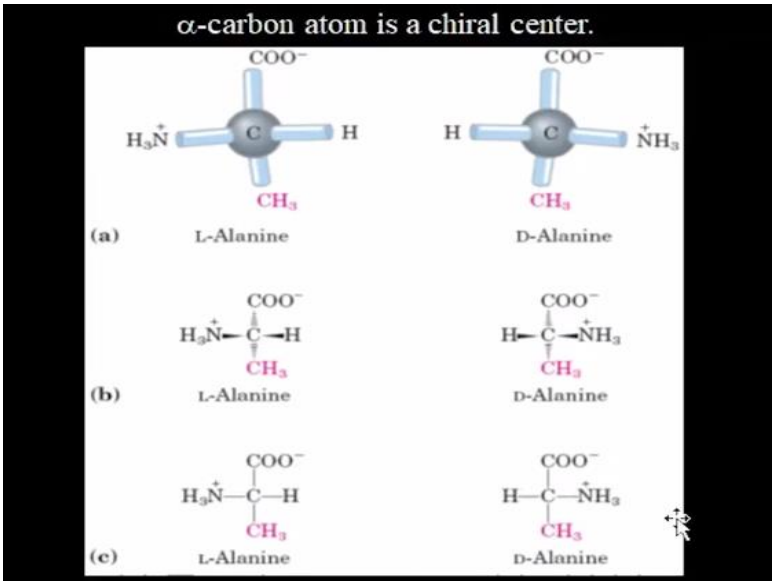

So, I already told you that alpha carbon is a chiral center. Let me make this little bit bigger, yeah so you can have a little bigger picture. So alanine has the slightly more complex group than glycine like instead of hydrogen here you have a methyl group. And so depending on the orientation here you could have L-Alanine or D-Alanine. It talks about the orientation of these groups around it.

Like for example if you see the relationship between these two if it is opposite orientation then it is one version and the other one is the other version. So L-Alanine has this orientation and D-Alanine has instead this orientation. So this is ball and stick and this is perspective diagram where you see this carboxyl group and methyl group are away from the plane of this slide and these two are projecting from the plane towards you.

So these two groups point away and these two groups point towards you. So, essentially these two are opposite to these two. So that is indicated by this angle here too. So the this tells you about little bit about the stereochemistry of amino acids and the other thing you want to pay attention is these groups can have different dissociation like for example here both are fully charged, so in this it is always fully charged.

But you can have a situation where this is protonated and this is not; so neither having a charge. And this also dissociated and having negative and this is negative, so various combinations of charges are possible. When the positives and negatives are equal as shown here, it is called as zwitterion.

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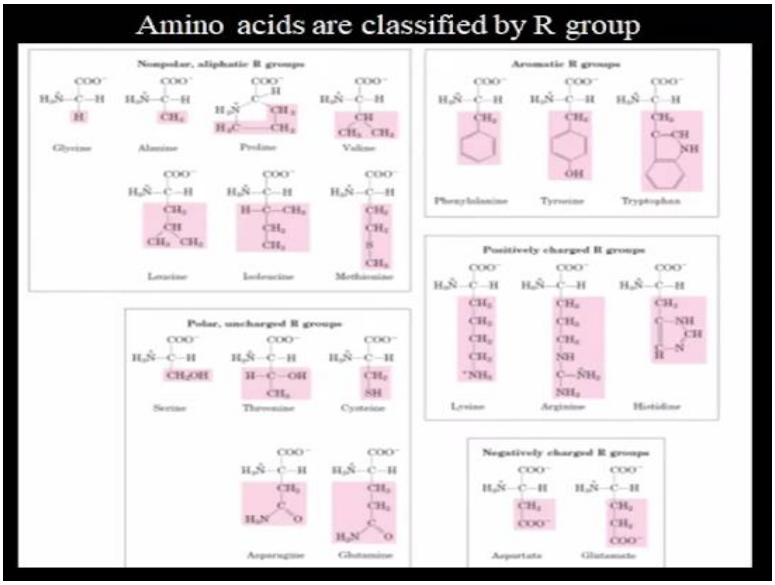

Zwitterion spelling will come somewhere, so I will not take the time to write it here, so where the acid and base charges like the net charge is 0 you call that as a zwitterion. So this slide shows you the different kinds of amino acids that are present. So I told you when we were discussing about proteins initially that proteins are made up of 20 different amino acids. So this table lists all the 20.

So you have a non-polar aliphatic groups like for example alanine and then to alanine you add two more methyl groups you get valine and you add one more CH to extend this chain then you get leucine, slightly rearrange it and you call it isoleucine and instead of one of the CH2 you have a sulfur then you could have methionine and then you make it as a chain with this amino group.

So this is basically an imino acid because there is no amino group because this amino group has become imino by this internal ring structure and that is a proline. So these amino acids including glycine do not have any charge or polar groups on them, so that is the characteristic of all of them and therefore they are the non-polar aliphatic amino acids. Then you can have aromatic ring instead of aliphatic ring like phenylalanine just the benzene attached to this methyl group.

Like you take alanine and then one of the hydrogens of the methyl group is replaced with benzene ring that is phenylalanine, have a phenol group instead like the hydroxyl group then you get tyrosine and instead you have a different aromatic ring like the indole ring you get

tryptophan. So, these are the aromatic amino acids, so of which one of them this is a polar because the hydroxyl group being present.

Then you have polar uncharged ones like instead of being totally hydrophilic you can have a hydroxyl group on the side chain like serine, threonine and cysteine you have an SH group here instead. So the methionine and cysteine are the ones that have a sulfur in them. So then you can have an amide group inside CONH2 asparagine and with one extra carbon then it becomes glutamine, so these are the amide groups.

So either you have an amide group or hydroxyl group or you have a thiol group so these are the polar ones. And then you have charged ones basic like lysine, asparagine and histidine, this is imidazole ring, guanidine and this is simply an amino group here. So these are the basic amino acids. And then you have the carboxylic acid containing ones aspartic acid and glutamic acid.

So these are the different side chains and based on the chemical properties of these side chains we have grouped them into 5 main groups. So if you go through them, these are not difficult to remember like if you just look at them once and pay attention to progressive increase in the number of moieties added and then if you know the functional group based classification you will readily be able to remember all the 20.

Like for example these 4 are related, aspartic acid this carboxyl group you convert into an amido group it is asparagine. This one you have to have one extra carbon then it is glutamic acid and then amide form of that is glutamine. So like this you will be able to easily remember. There is no excuse for not knowing the 20 amino acid structures and calling oneself graduate in biotechnology or biochemistry or biology or whatever.

Anything starting with bio and if you have done a 4-year degree you should be able to write all the 20 amino structures anytime, even in the middle of sleep if someone asks you to write you should be able to write. So, I will stop here today and we will continue our discussion on proteins in the next class.