Introduction to Complex Biological Systems
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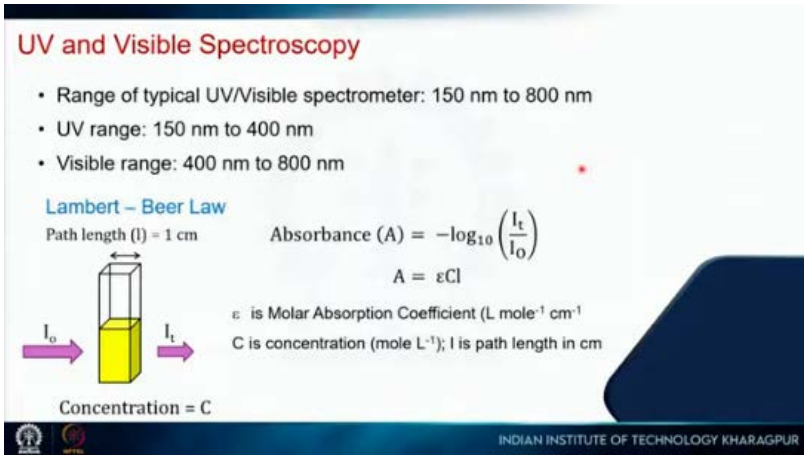
Lecture 56
Characterization methods

Welcome to the final week of this course on introduction to complex biological systems. So, this week, I am going to talk about some biophysical and biochemical methods, which are commonly used in biological experiments. So, today I will start with UV-visible spectroscopy, and then I will also talk about gel electrophoresis methods. So, what is UV-visible spectroscopy?

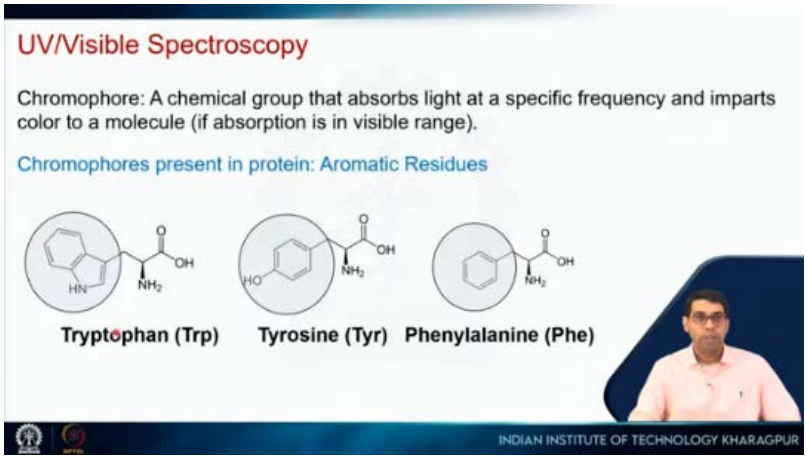


The range of a UV-visible spectrometer, which is the instrument used for this spectroscopy, is typically 150 nm to 800 nm. The UV range is 150 to 400 nm, and the visible range is 400 nm to 800 nm. Now, it turns out that Lambert-Beer's law states that if you have a solution, let us say this is your protein solution. You incident light at a particular wavelength, then some of it will be absorbed, and the rest is transmitted.

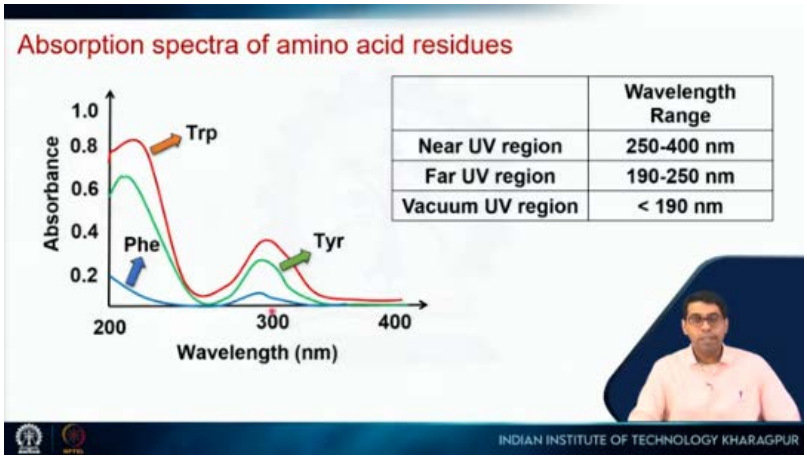
So, you can calculate the absorbance, the amount of light that is absorbed, using this formula, which is $-log_{10}\left(\frac{I_t}{I_o}\right)$. So, this is the initial, and this is the transmitted one. Since this is less than this, the log of this will be a negative number, so we can turn it into a positive number by adding a negative sign here. Now, this absorbance depends on these three things: epsilon(ε), which is the molar absorption coefficient of the molecule; so we will see how we can determine this C is the concentration of your sample, and L is the path

length, which is this distance that the light passes through the sample; typically, we use a cuvette like this, and the path length will be 1 centimeter.

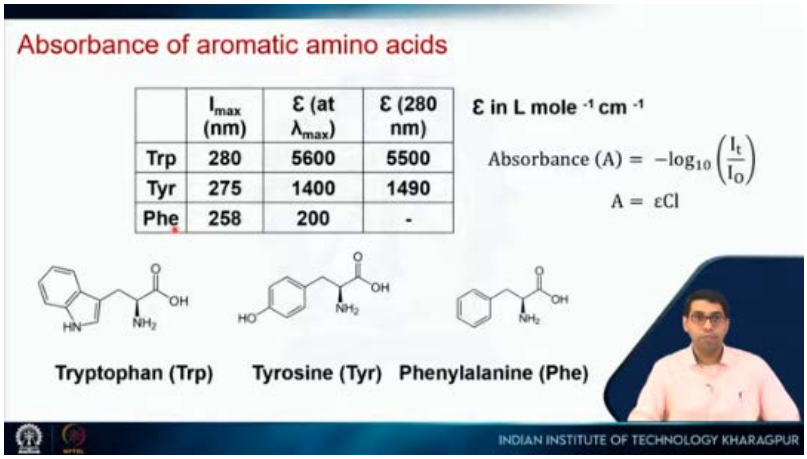
So, if we can measure the absorbance, and if we know the epsilon(ε) and we also know the path length, we can calculate the concentration. So, this UV-visible spectroscopy, one of the most common uses, is to determine the concentration of your sample. So why is there absorbance? It turns out that there are amino acid side chains, typically these aromatic side chains, which are chromophores. What is a chromophore?



A chromophore is a chemical group that absorbs light at a specific frequency and imparts color to the molecule and, of course, this color will be visible if it is in the visible range. So, the chromophores that are present in proteins are these aromatic residues. So, tryptophan side chain, tyrosine side chain, and phenylalanine side chain. These are the spectra.



So, this is your UV-visible. This is mostly the UV range that we can see. So, they absorb in this UV range. So, this is phenylalanine; you can see that there is not much absorbance. This is tyrosine; it is much better, and this is tryptophan, which is even better. Now, the side chains we are mostly interested in are in this particular region, and they absorb in this near UV range. So what are the maxima? So you see that there is a maximum wavelength at which the intense absorbance is the maximum; so this will be called λmax, and you can see that this λmax slightly varies between these three different chromophores. The λmax for these 3 chromophores are noted here. So, for phenylalanine it is 258 nm, for tyrosine it is 275, and for tryptophan it is 280. So, these are very close to each other.



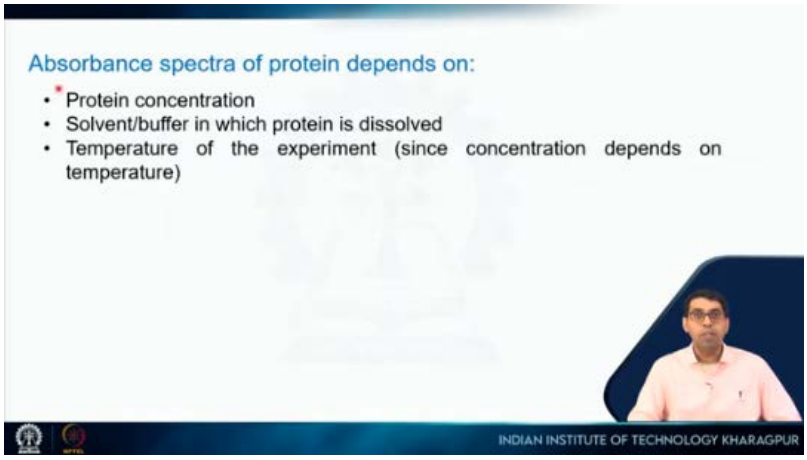
	I_{max} (nm)	Ɛ (at λ_{max})	Ɛ (280 nm)
Trp	280	5600	5500
Tyr	275	1400	1490
Phe	258	200	-

So, typically what we do is we do our UV visible spectroscopy or the UV spectroscopy. So, we measure at 280nm. At 280nm, the absorbance or the epsilon(ε) for tryptophan is this, and for tyrosine it is 1490 and 5500 for tryptophan. Now, let us assume that you have a protein, you know the sequence of the protein, and you can see that for phenylalanine there is not much. So, we can neglect phenylalanine.

So, let us say your protein has 2 tryptophan and 1 tyrosine. So, from this you can roughly calculate the epsilon(ε) of your molecule of your protein sample. So, since there are 2 tryptophans you multiply this by 2. This will be $10000 + 1000 = 11000 + 1490 = 12490$ will be your epsilon(ε). Now, you measure it using a UV-visible spectrometer; you will get the absorbance, and you know L is 1 centimeter. So, you can calculate your C, the concentration of your protein sample and we need to know the concentration of this protein

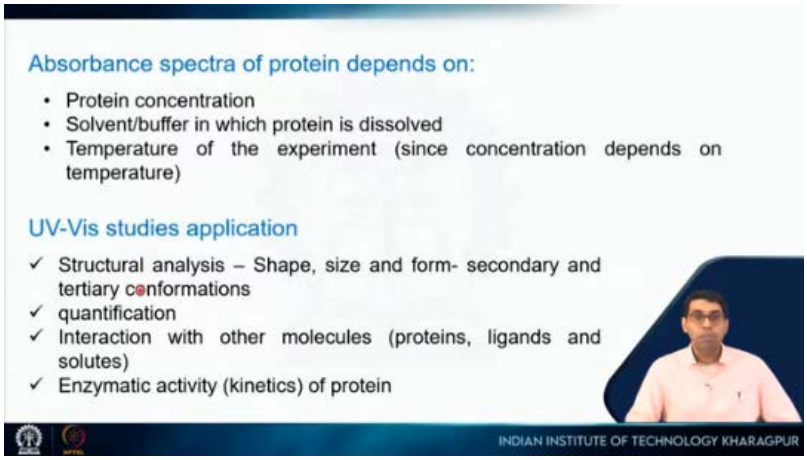
sample for various experiments. If you want to do some protein-ligand interaction, you need to know the concentration of your protein.

If you want to do enzyme kinetics, you need to know the concentration of your protein. So, there are all these different uses for protein concentration. This is one of the basic things that you want to know. So the absorbance spectra of the protein depend, of course, on the protein concentration. It can also depend on the solvent buffer in which the protein is dissolved. So, what we normally do is use something called a blank. So, you put the solvent or the buffer in which the protein is dissolved.

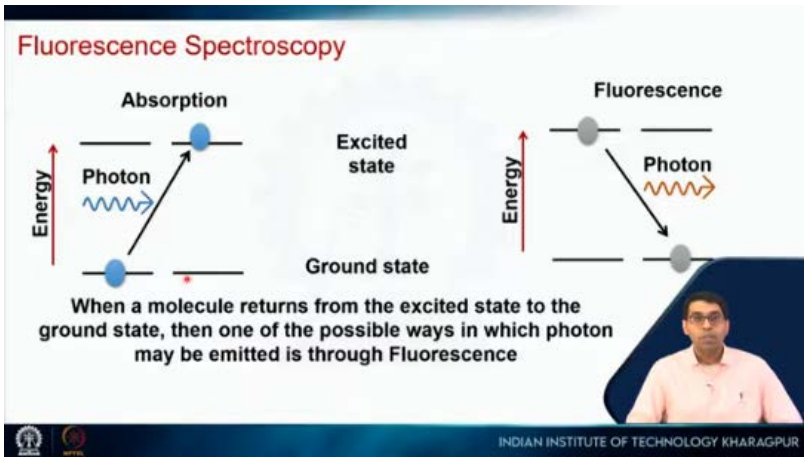


Just the solvent without the protein; you measure the absorbance, and then you add the protein to it and measure the absorbance again. So, then you can subtract the absorbance that is because of the buffer. It can also depend on the temperature of the experiment. Typically, we do these experiments at room temperature. But, depending on your application, you may want to do it at a higher temperature or at a lower temperature.

So, the concentration that you measure will depend on the temperature also. What are the applications? We can get structural information. So, for example, the shape, size, or even secondary and tertiary structure information, depending on how you do the experiment. So, we will see in more detail when I talk about CD spectroscopy in the next lecture, where you will see that you can get detailed information about the secondary structure of your protein from a CD experiment.

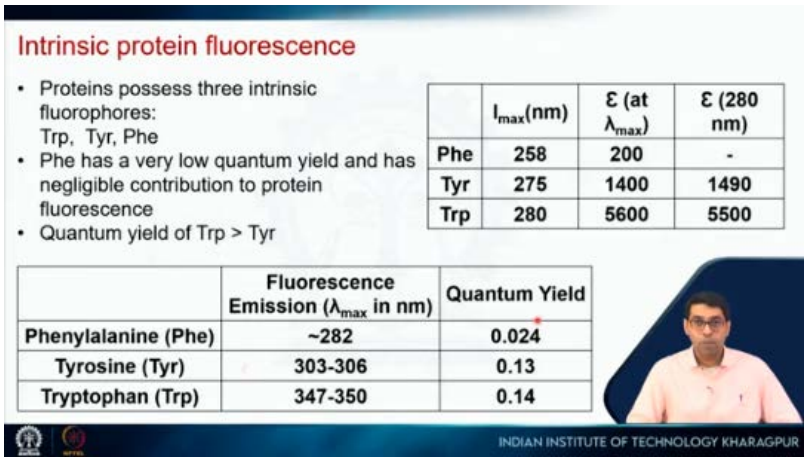


We have already seen that we can use UV-visible studies to quantify the amount of protein. So, it is very useful for quantification. We can also use this for interaction studies and as I said, we can also use this for kinetics. So, apart from that, the same chromophores can also be used for fluorescence studies.



So, what is fluorescence? You have your chromophore; you can give energy. So, we are incidenting some light, and it goes to an excited state. Now, it can come down through various radiative processes. One of those is fluorescence and you measure whichever photon is emitted. It typically turns out that you excite at a shorter wavelength and you emit at a longer wavelength because this energy will not be exactly the same as this energy. So, you will lose some of the energy, and then you get fluorescence. So typically, there is a shift in the wavelength to a greater wavelength and that is exactly what we use. Now again, you will see that phenylalanine is not very good because its quantum yield is very low, but tyrosine and tryptophan have decent quantum yields. So, we can actually use

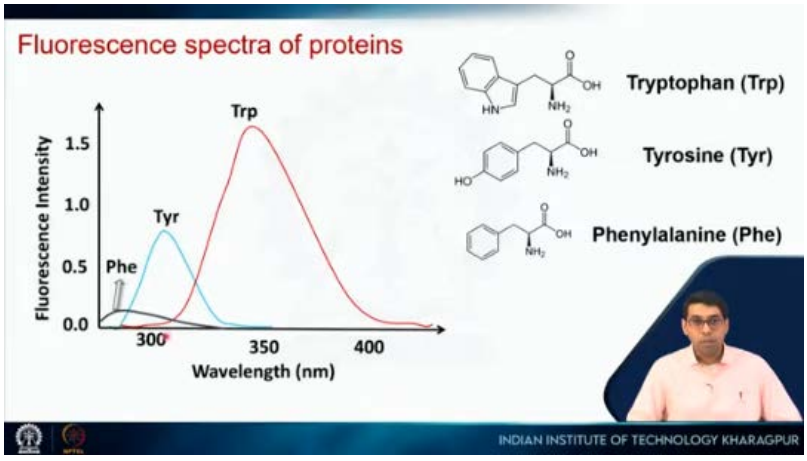
tyrosine and tryptophan for our fluorescence studies. Again, the epsilon(ε) values are written here.

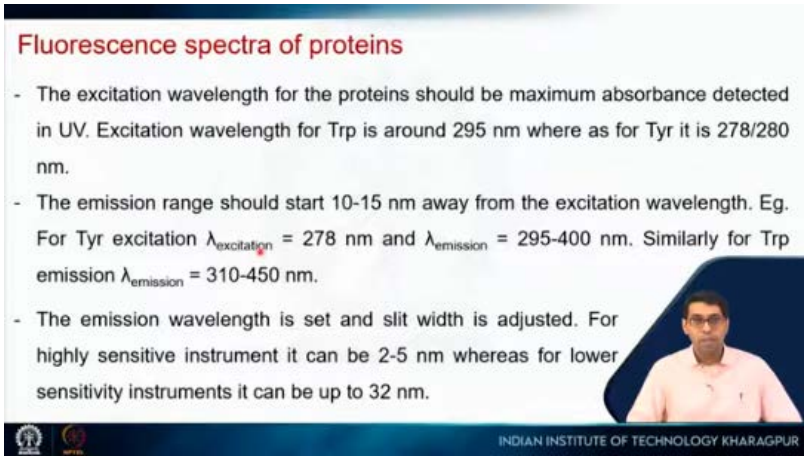


	l_{max}(nm)	Ɛ (at λ_{max})	Ɛ (280 nm)
Phe	258	200	-
Tyr	275	1400	1490
Trp	280	5600	5500

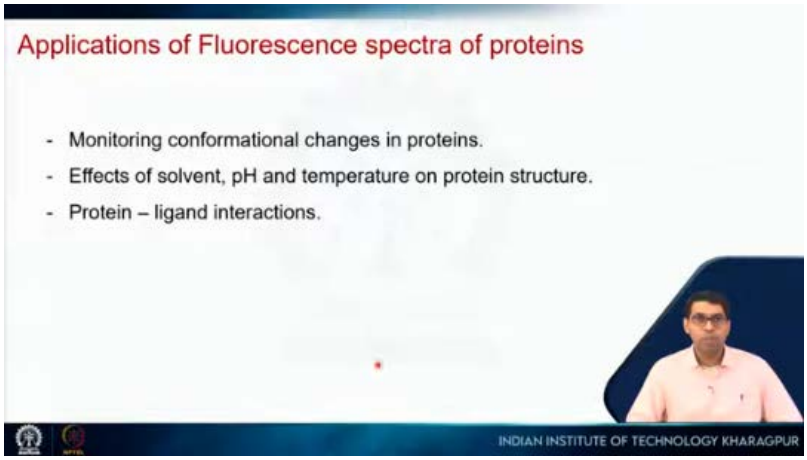
	Fluorescence Emission (λ_{max} in nm)	Quantum Yield
Phenylalanine (Phe)	~282	0.024
Tyrosine (Tyr)	303-306	0.13
Tryptophan (Trp)	347-350	0.14

So, if we look at the fluorescence of these molecules, this is phenylalanine; you can see it is very low. Tyrosine has a decent signal, and tryptophan has a much better signal. So, we can typically use tyrosine and tryptophan for our fluorescence studies. So, just like the UV spectrometer, you can use a fluorimeter to observe the fluorescence spectra of proteins.



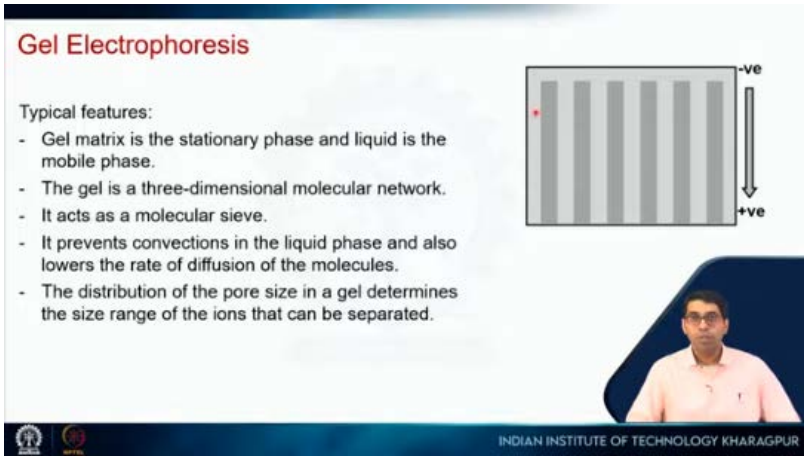


The excitation wavelength for the protein should be the maximum absorbance detected in UV. Now, this excitation wavelength of tryptophan is around 295nm, whereas for tyrosine, it is around 280nm. So, this is the wavelength at which you will excite your protein. So, if you want to look at tryptophan, you excite it at 295nm; if you want to look at tyrosine, you excite it at 280. The emission range should be at least 10 to 15 nm away; otherwise, there will be overlap, so ideally, it should be 10 to 15 nm away. Now, for tyrosine, if you excite at 278nm, its emission will be around 295nm to 400nm, so this is more than 15 nm away, which means that the instrument can detect it efficiently. Similarly, for tryptophan, if you excite it at 295nm, you will observe the emission in the range of 310nm to 450nm. It turns out that we cannot excite exactly at a particular wavelength because we use certain monochromators. What they do is that modern instruments are highly sensitive and can use this very narrow range for excitation, 2nm to 5nm. But if you have an older instrument, it can be as broad as 32nm, which will overlap. So, modern instruments are much better in this regard.



The application is very similar. So, you can monitor conformational changes in proteins. For example, if you have a tryptophan that is buried inside the core of a protein, it will have certain fluorescence, and if it is exposed because of some conformational change, it will have a different fluorescence. So, you can observe this change in fluorescence. It can be a change in intensity or it can also be a change in the wavelength of the emitted fluorescence, and from that, you can determine conformational changes. The same conformational changes can also happen as a function of solvent pH or temperature. For example, you might want to look at the denaturing of your protein as a function of temperature. As you increase the temperature, your protein unfolds. So, a tryptophan side chain, which was buried because it is hydrophobic, now gets exposed to the solvent, and its fluorescence changes.

Similarly, we can look at protein-ligand interactions. If there is a tryptophan or a tyrosine on the binding interface, which is typically the case, then its fluorescence will change depending on whether the protein is free or bound to a ligand. Now let us come to gel electrophoresis. Just like UV-visible and fluorescence spectroscopy, gel electrophoresis is something that, if you are working in a biochemistry lab, you will use almost on a daily basis. So, the gel electrophoresis that I am going to talk about is SDS-PAGE gel electrophoresis. You have already seen agarose gel electrophoresis for DNA. So, what are the typical features?



The first thing you need is a gel matrix, which is the stationary phase. So, the gel matrix will look something like this. This can be made up of polyacrylamide or agarose, and there is a liquid which is the mobile phase. So, the liquid will move from one end to the other end of the gel. Now, this gel is a three-dimensional molecular network.

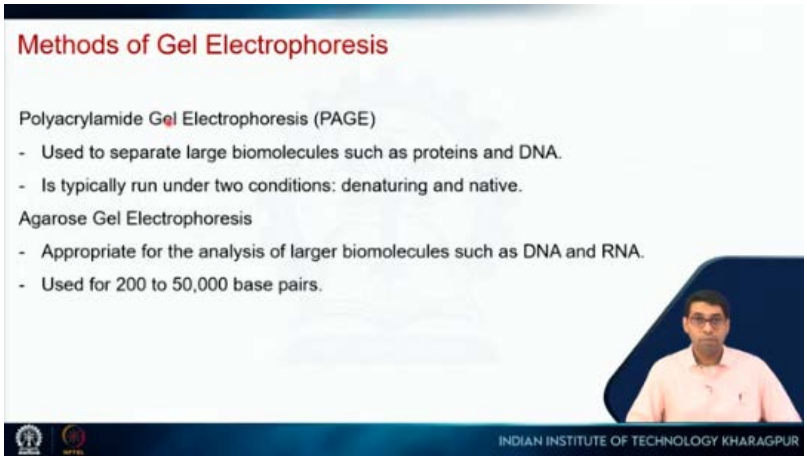
So, there is a mesh and through those small there are small pores through which your molecule will pass through. It turns out that typically for SDS PAGE or polyacrylamide gel, the thickness of this gel will be around 0.5 millimeters and or maybe up to 1 millimeter for agarose, this is around half a centimeter or 5 millimeters. Now this gel matrix acts as a molecular sieve and it has several functions, so it prevents convections in the liquid phase, so your liquid will not move like this; it will just move in a very streamlined fashion, and it also lowers the rate of diffusion of the molecules. The distribution of the pore size in a gel determines the size of the ions that can pass through.

So ultimately you want to separate your molecules according to size and that will be determined by the pore size of your gel. So what we do is we apply charge, there will be a negative charge in one end and there will be a positive charge in the other end. So in case of DNA, we have seen the DNA molecules are negatively charged, so they will move towards the positive end. In case of proteins, what we will see is that we impart this negative charge on protein by adding SGS. So the protein molecules will also be uniformly negatively charged and they will also move from this end to this end.

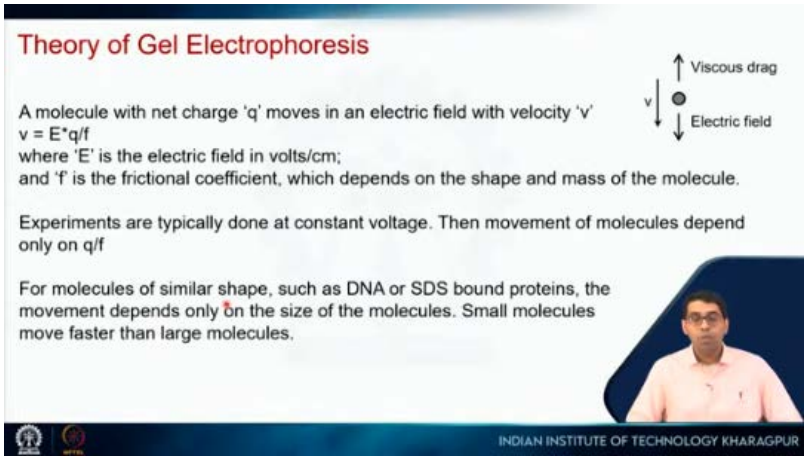
So these are the typical lanes. So we will create these lanes in the gel so that the protein molecules, so if you put your sample here, it will only move in this lane. There will be no

diffusion from one lane to the other lane. That is what this matrix prevents. So, there are two major matrices that we use: polyacrylamide gel and agarose gel.

So, polyacrylamide gel is used to separate large biomolecules like proteins and DNA and it typically runs under two conditions: denaturing and native. So, today I am going to discuss only denaturing gel and I will talk about native phase when I talk about protein ligand interaction. You have already seen agarose gel electrophoresis; it is appropriate for even larger biomolecules such as DNA and RNA, so if the size of your DNA is 200 base pair to fifty thousand base pair, you can use agarose gel electrophoresis here the pore sizes are much bigger. But if your DNA is smaller, then you will use polyacrylamide gel electrophoresis. We will see that we will use polyacrylamide gel electrophoresis under native conditions to look at protein-DNA interaction, where the DNA size will typically be less than 200 base pairs.



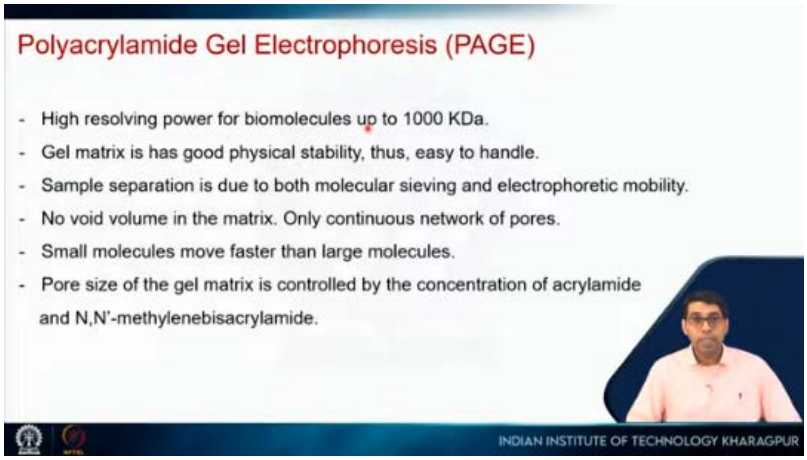
So what is the basic principle? It turns out that we have a charged molecule which is running or which is moving in an electric field. So this is the situation that we have. We have a protein molecule or a DNA molecule. It is negatively charged.



It has overall a negative charge. So it will move in the electric field from the negative end to the positive end but it passes through this different pore size. So, there will be a viscous drag which acts in the opposite direction, and this viscous drag will be proportional to the size of the molecule. So, this frictional coefficient depends on the shape and size of the molecule.

The velocity at which your molecule moves depends on the electric field E, where q is the charge and f is the frictional force. Now, typically, experiments are done at constant voltage, which means your E is constant. So, this is volts per centimeter. Then the movement depends only on q by f. Now, if we think about DNA, it has a similar shape. So, all DNA will be rod-like, and the charge distribution will be very similar because the charge comes from the phosphate backbone.

The same thing happens for SDS-bound proteins also. So when SDS, which has a negative charge, binds to your protein, it will denature the protein and make it a rod-like shape. It will look very similar to DNA. So you will have a rod-like shape, and the charge will be uniformly distributed on it. So the result is that the movement of these molecules depends only on the mass of the molecule because you have made the shape also uniform. It will depend only on the mass of the molecule. Now, smaller molecules will move faster, and larger molecules will move slower because smaller molecules can pass through small pore sizes and large pore sizes, whereas larger molecules can move only through the larger pore sizes. So that is how we can separate our molecules according to size. So, these are some of the features of polyacrylamide gel electrophoresis.

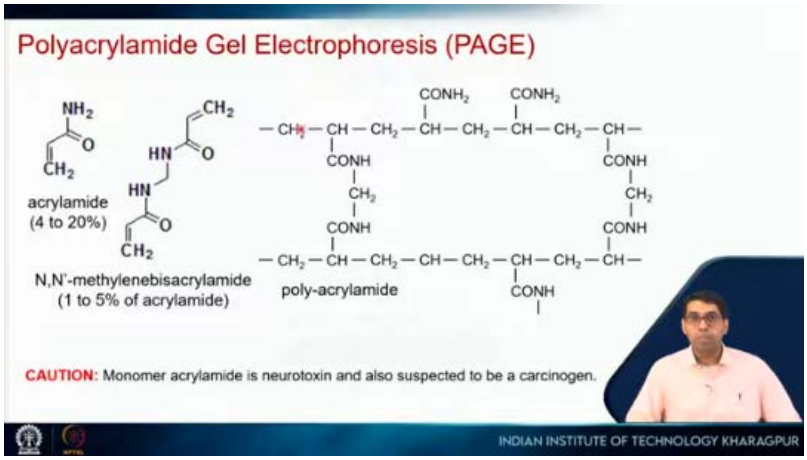


It has a very high resolving power for biomolecules up to 1000 kilodaltons(KDa), so 1 megadaltons. The gel matrix has very good physical stability, which means that it is easy to handle. So, typically what happens is once you run the gel, which is between two glass plates, you have to remove the glass plates, take the gel out by hand, and then stain it. Then you can see the proteins. So, those things need good physical stability and are easy to handle, and that is what we get with this PAGE.

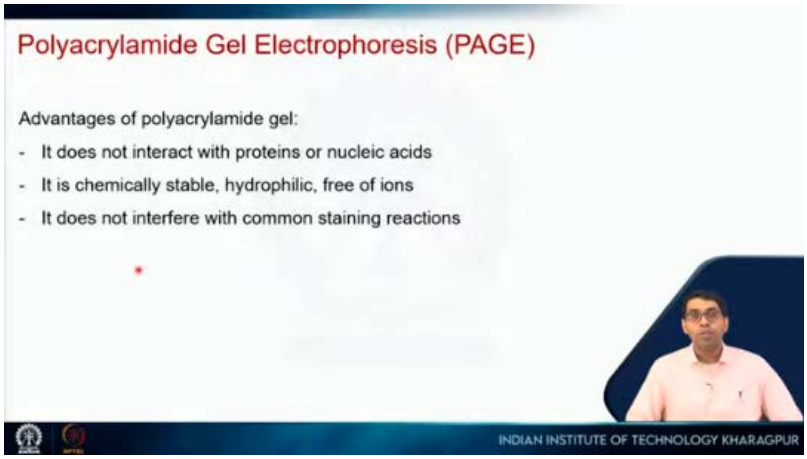
Sample separation is due to both biomolecular sieving and electrophoretic mobility. That we have already discussed. It turns out that there is no void volume in the matrix. So, it is a continuous matrix, which means that all the molecules will have to pass through these different pores. They cannot bypass passing through these pores.

That is why we can separate them according to their size. Small molecules will move faster than the larger molecules. The pore size of the gel matrix is controlled by the concentration of the monomers that we use to make the gel matrix. You will see that there are two types of monomers that we use. So, one is acrylamide.

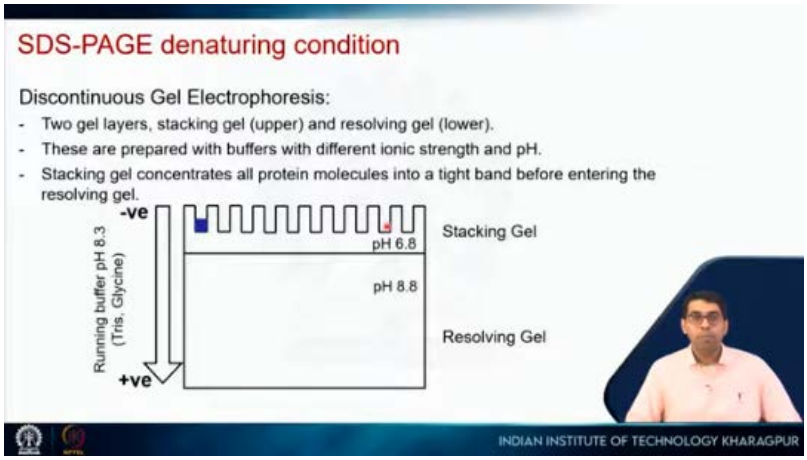
We use a certain percentage of this acrylamide, 4% to 20%, and then there is this bisacrylamide. So, these are the monomers and we mix them in a certain ratio, so that we can get the polymer. This acrylamide will form this linear polymer chain like this. However, a linear polymer chain will not form a gel matrix because you have to connect them in three dimensions. So, that connection is done by this bisacrylamide.



When you add them, so you have more of this, you have less of this. We add them in a certain ratio and then initiate the polymerization, and we get this network of these polymers which form this three-dimensional gel matrix. Now, you should remember that monomer acrylamide monomeric form is neurotoxic and it can also be a carcinogen. So, we have to be very careful when handling these molecules; once it forms a polymer, it is fine, but the monomeric form is toxic.



Again, what are the advantages? It does not interact with the protein or nucleic acid. So if it interacts, then of course you have a problem. So it is very inert to your sample. It is chemically very stable. It is hydrophilic and it is free of ions and it does not interfere with common staining reactions. So it is something that is very useful and again, you will see that in a biochemistry lab or in a molecular biology lab, these experiments are done almost daily. So this is how your gel looks. So what we are going to do is use SDS-PAGE electrophoresis.



So PAGE is polyacrylamide gel electrophoresis, and SDS is the molecule that we are going to add. So it will denature your protein and also give a uniformly negative charge to your protein. Now the setup will have two gels. It is not just one gel. There are two gels.

This one is called a resolving gel. So the actual separation happens here, and this one is called the stacking gel. I will explain what the purpose of this stacking gel is and it turns out that we use three different buffers, one to make the resolving gel, which is at pH 8.8.

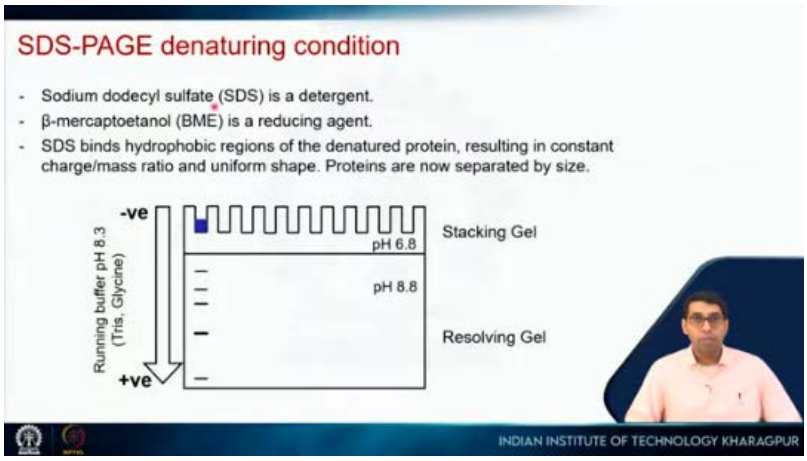
One is to make the stacking gel, which is at pH 6.8, and the third one, which is the running buffer. So, this will be the buffer that will run through this. So, that's the mobile phase, and it is at a pH of 8.3, and it will be made up of Tris and glycine. So, these are prepared with buffers of different ionic strength and pH, as shown here. The stacking gel concentrates all protein molecules into a tight band before entering the resolving gel.

So, as I told you, the resolving gel, as the name suggests, is where the protein molecules will be resolved. So, let us say you have a mixture of 10 different protein molecules. So, you want them to be resolved, according to their size, and that will be done by the resolving gel. But typically, we have these lanes.

So, what you see here are the lanes, and this is where you load your sample. So, when you load your sample, there will be a certain volume. So, let us say this is a 10 microliter sample; if you load 20 microliters, it will go almost up to the top. You can see that your protein molecules will be distributed in this particular volume. So, there will be molecules at the bottom and molecules at the top.

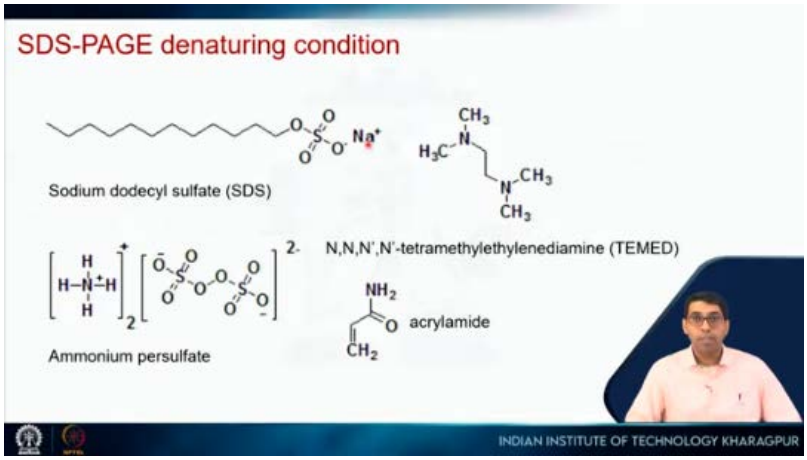
So, now if they enter into the resolving gel like this, then the one which is at the top will run behind, and the one which is at the bottom will run ahead. So, what you will get is diffusion. So, your protein molecules will diffuse, and you will get a diffused band, and it will be very difficult to analyze. So, what we want is to concentrate all of these in a very tight band before the proteins enter into the resolving gel.

This is like a race. So, you want all your athletes to be on the same starting line. So, this is the starting line. So, all 10 protein molecules should be on the same starting line and then they should start running, which is in the resolving gel, and they will run at different speeds. So, they will get separated according to their size because the speed will depend on the size. So, that is the purpose of the stacking gel to bring all the protein molecules, which are distributed in this length, let us say, into a very tight band at this point and then you can get a nice separation. So, what we have is sodium dodecyl sulfate, which is a detergent; you can also use beta mercaptoethanol or BME, which is the reducing agent. So, it will depend on your sample; if your protein has cysteines, you will add this, and if it does not, then maybe you can skip it.



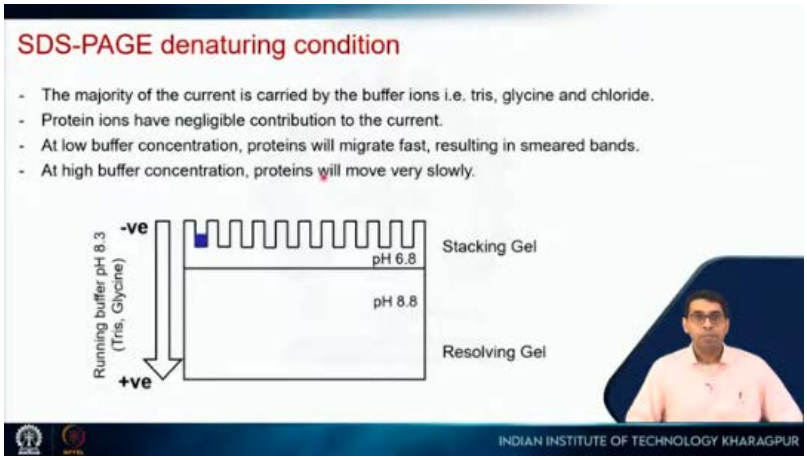
So, SDS will bind to the hydrophobic region of the denatured protein, resulting in a constant charge-to-mass ratio and uniform shape. So, now proteins are separated by size. It turns out that typically one amino acid will bind to SDS molecules. So, this is your sodium dodecyl sulfate; it has a negatively charged group and then it has this long hydrophobic chain, which binds to the hydrophobic part of your protein or hydrophobic

amino acids of your protein. You have already seen acrylamide and bisacrylamide, which form the polymer.



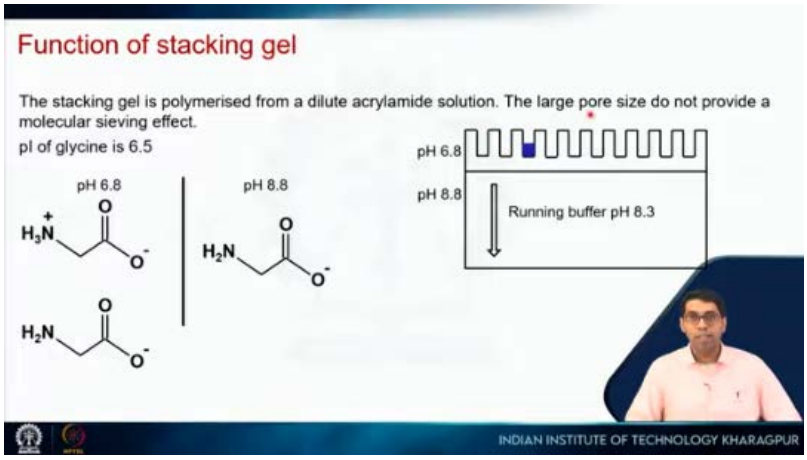
However, the polymerization reaction is a free radical reaction, and this free radical is supplied by ammonium persulfate. This breaks and forms a free radical. However, ammonium persulfate is not something that can transfer this free radical very efficiently. So, we need something called an initiator. This is N,N,N′, N′-tetramethyl.

So, there are four methyl groups, ethylenediamine. This free radical forms a free radical on this, and that can very easily or efficiently transfer the free radical onto the acrylamide and bisacrylamide, thus initiating the reaction. This is called the initiator; this is the free radical provider, and you have the acrylamide and bisacrylamide. So, typically we mix all of them, pour them between two glass plates, wait for around 15 to 30 minutes, your gel is formed, and then you can start your experiment. So, this is again your gel; the majority of the current is carried by the buffer ions.



Now, in the buffer there will be Tris, there will be glycine and there will be chloride, and we will see the function of these ions in the next slide. Protein ions have a negligible contribution to the current because these are big molecules, and your concentration is very low compared to this concentration. So, the majority of the current is carried by these ions. But you have to be very careful about your buffer concentration. There are typical recipes and if you follow them everything will be fine.

So, if by mistake you have low buffer concentration, then the protein will start moving fast because the current will be carried by these protein ions. So, then you will get smeared bands. On the other hand, if you have high buffer concentration, your proteins will move slowly. So, you have to use the right conditions to do these experiments. So let us see how the stacking gel brings all these protein molecules into a very tight band just before entering the resolving gel.



So there are two ions, you have glycine and you have chloride ions. If you look at glycine, the glycine has these two charge groups, the carboxylic group and the amino group. Now the PI of glycine is 6.5. You see the pH, it is around 6.8, slightly above the PI of your glycine molecule. So, what happens is that at this pH of 6.8, most of the glycine molecule will be present in its zwitterionic form, which is the neutral form, and a very small fraction will be present in its negatively charged form.

So, in this stacking gel, your glycine is mostly in this form and a very small amount in this negatively charged form. You also have chloride ions which are negatively charged; they are very small. So, they have a very high charge to size ratio. So, charge density will move very fast. The chloride ions produce a front chloride front.

So, that is the one which is moving the fastest, and this glycine ion will form a very slow-moving front in the back. It will be so; this will run slower than this. Now, it turns out that we are doing our experiment at a constant voltage and V equals to IR. So, the region where these glycine ions are moving. Since you have very few negatively charged ions, your current becomes low.

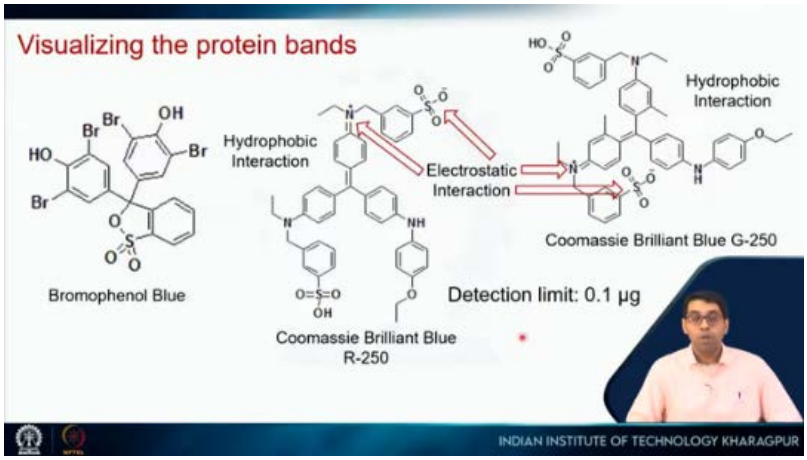
So, there are fewer ions which are carrying the current, but the current is constant throughout this. So there is no accumulation of charge. It means that since you have lesser ions your resistance becomes high. To maintain the same current, your local voltage will also become high because the resistance has increased, so the region where these glycine ions are moving, the local voltage increases and that increase in local voltage will result in faster moving of the protein molecule.

So the protein molecules will see this higher voltage. They will move fast and once they reach; this is your protein molecule. Once they reach these chloride ions, your resistance here is low. So they will stop just in front of these chloride ions.

This is what happens when all the protein molecules pass through this and they will all come and reach this chloride ion front and stop there so all the protein ions are concentrated right here. So you have chloride ions, protein ions, and glycine ions. These are all moving together, forming a very tight band.

So all your protein molecules are concentrated into a very thin region, which is the case here. Now, the moment all these ions pass into the running buffer, what happens? You have a pH of 8.3. So at that pH, all your glycine ions will mostly be in this negatively charged form, so now they will start moving faster than your protein ions because they are much smaller in size. So, you do not have this effect in the resolving gel. So, everything, the chloride ions and the glycine ions will move faster as they are supposed to. The protein ions will now move according to their size, and we get this resolution of your protein ions according to their size. Then we will see different bands.

So, how do we see these different bands? So, for that, we have to stain them using certain dyes. When you make your protein sample, you will add something called bromophenol blue, which I am showing by this blue color. So, this bromophenol blue is added just for better visualization because most proteins will be colorless. So, you know you are adding it properly, and it also forms a dye front that runs slowly, something in the range of around the 5 kilodaltons size. Even though it is small, it does not have a very high charge.



So, it will run slowly. So, you can tell how long you have to run the gel. So, it serves this dual purpose, but once it is done, you have to visualize your proteins. So, you take out your gel. You soak it in a solution which has Coomassie Brilliant Blue, and it comes in two different forms: it can be R-250 or G-250. This has negative and positive charges and a huge hydrophobic moiety, so all of this will stick to your protein. This is a very intense blue color, so you can see the protein bands.

So, this is a typical apparatus. This is the gel electrophoresis apparatus. Typically, SDS-PAGE or polyacrylamide gel electrophoresis is done vertically. So, this is the setup. You have electrodes.

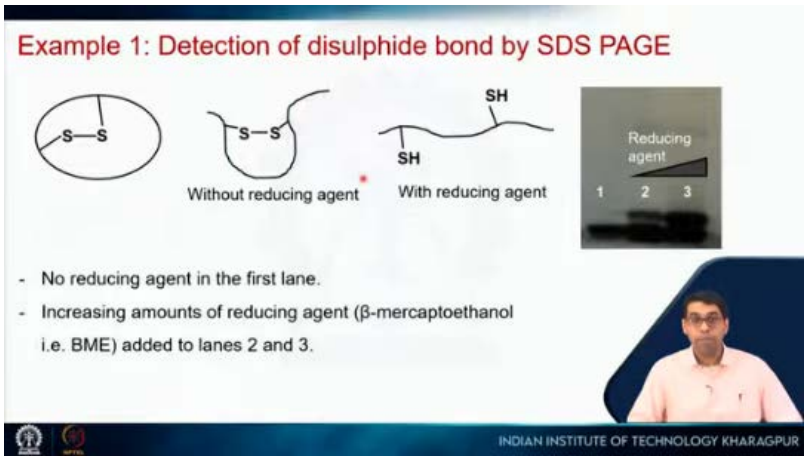
So, the red one is the positive one, and the black one is the negative one. So, you connect them correctly, and then you start, and there will be a constant voltage supplied by this power source, and you will have your gel electrophoresis. So your polyacrylamide gel will be between two glass plates. You can see there are two glass plates, and then that glass plate is assembled here, and then the whole thing goes into this. You pour a buffer here, and that will be your running buffer, and the electrodes you can see here. So, once you run, you stain it with Coomassie Brilliant Blue. Normally, it will look blue, but if you take a picture using some gel documentation, it will just convert it into a black and white image. So, to see your protein bands, you will see your protein bands like this.

Now, to know what is an approximate size, what we do is we use something called a molecular mass ladder. So, it is a mixture of proteins which have known molecular masses. So, this is a 10 kilodaltons protein size, it is a 15 kilodaltons, 20, 25 kilo Dalton, and so on and so forth. So, if your protein is running here, you can tell that my protein is somewhere between 10 to 15 kilodaltons and from your sequence, you can calculate its actual protein size or protein mass, and you can compare that my protein is 13 kilodaltons and it is running here.

So, this is my protein. So, you are not just looking at the purity of your protein or seeing whether the protein has the correct size; you can also do some other interesting

experiments. For example, let us say your protein forms a disulfide bond like this, and you want to confirm. So, you know that there are two cysteines, but you do not know whether these two cysteines form a disulfide bond or not. So, what you do is you can run your sample.

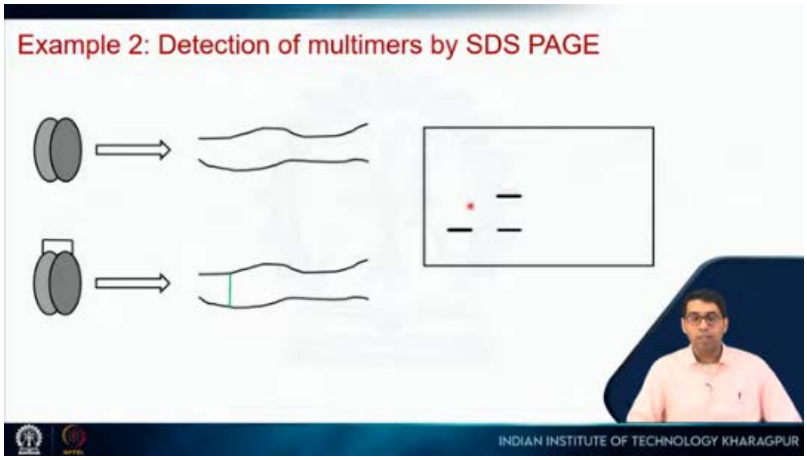
So, you run your gel where in one sample you do not add any reducing agent. Then in the second sample you put some reducing agent, in the third sample you add more reducing agent. So now what happens when you denature your protein if you do not put any reducing agent and if the two cysteines are forming a disulfide bond, it will look like this. So SDS will not break this bond. So what you will have is a more compact structure of your protein. But, if you add a reducing agent, it will be a more open structure. So, this will move faster, this will move slower. When you do not add any reducing agent, this will move faster and this will move slower because this is a more compact structure. So, when you do not add any reducing agent, you get this band, but when you add a reducing agent, some of your molecules will be reduced. So, you will start seeing a second band because it is longer. So, it will move slower, and as you keep on adding more and more reducing agents, this band will become thicker and thicker, and this will become smaller and smaller. So, just by doing this experiment, you can tell that yes, there are these two cysteines forming a disulfide bond.



Now, suppose there are two cysteines which do not form a disulfide bond, then under these two conditions, in the presence of a reducing agent, you will get this; in the absence of a reducing agent, you will also get this because they are not forming a disulfide bond. So,

you will not see a second band when you add a reducing agent. So, from this simple experiment you can tell whether the two cysteines present are forming disulfide bonds or not. Similarly, if your protein forms multimers, let us say it forms a dimer.

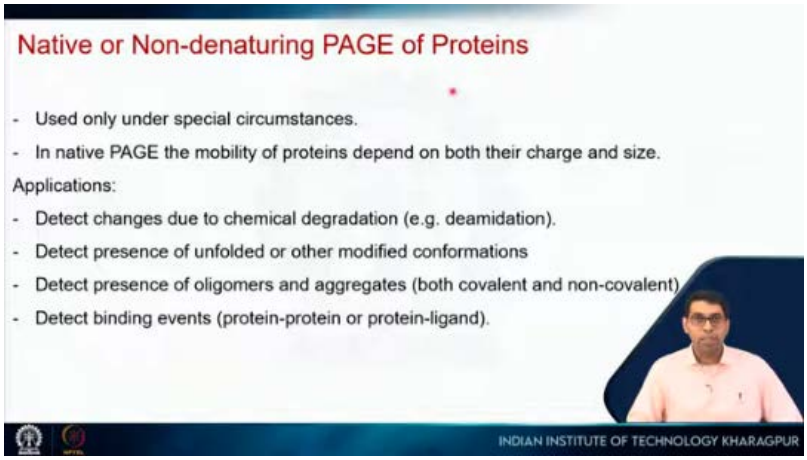
If you denature it, then the two polypeptide chains will come apart and they will run at the same position. But what you can do is add certain cross-linking agents like glutathione. So you can add a cross-linking agent. So you use your protein at a very low concentration so that there is cross-linking only between the two monomers of the dimer, and now if you denature it, it will look something like this. So now if I run my protein sample, I will get some molecules where you do not get this cross-linking, so you will get the monomers, and there will be some protein molecules where you will get cross-linking and you will get a dimer. So, again you can do this experiment as a function of concentration. So, you keep on increasing the concentration of your cross-linking agent.



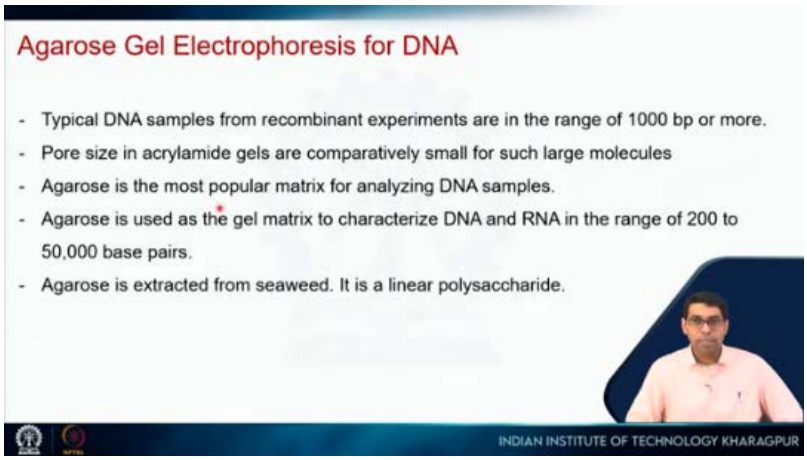
So, you will see more and more dimer formation and less and less monomer. That will again confirm that your protein is a dimer and again you can tell this is a monomer and this is a dimer from the molecular mass ladder. So, let us say this monomer is 15 kilodaltons. So, it will show up where a 15 kilodaltons ladder protein is and this will be 30 kilodaltons.

So, it will show up where a 30 kilodaltons ladder protein is or maybe between 25 and 35 kilodaltons ladder proteins. So, from this you can tell that yes, it is forming a dimer. If it forms a tetramer again, you can do the same thing, and from this, you can figure out that yes, my protein forms a tetramer. Polyacrylamide gel electrophoresis can be done in native

conditions also, and I will discuss this in more detail in the lecture where I talk about protein-ligand interaction.

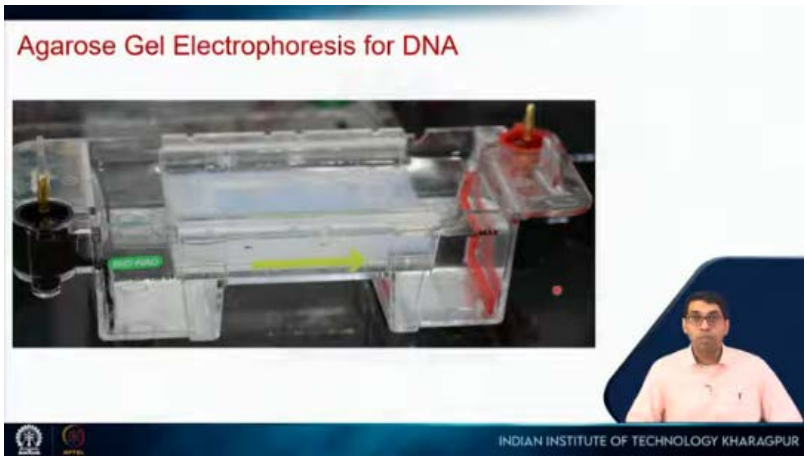


So it can be done only under special circumstances in native page; mobility of protein depends on both their charge and size because we have not denatured it. So, applications can be used to detect chemical degradation, for example, deamination. It can detect the presence of unfolded or other modified conditions. So, if the protein is unfolded versus folded, it will migrate differently. If you have oligomers or aggregates, you will see multiple bands and we can also look at protein-protein or protein-ligand interactions. So, this is just a brief recapitulation of agarose gel electrophoresis for DNA. So, here the experiments are done for much larger molecules. So, the DNA is 100 base pairs or longer. Pore sizes in acrylamide are comparatively smaller for such large molecules.

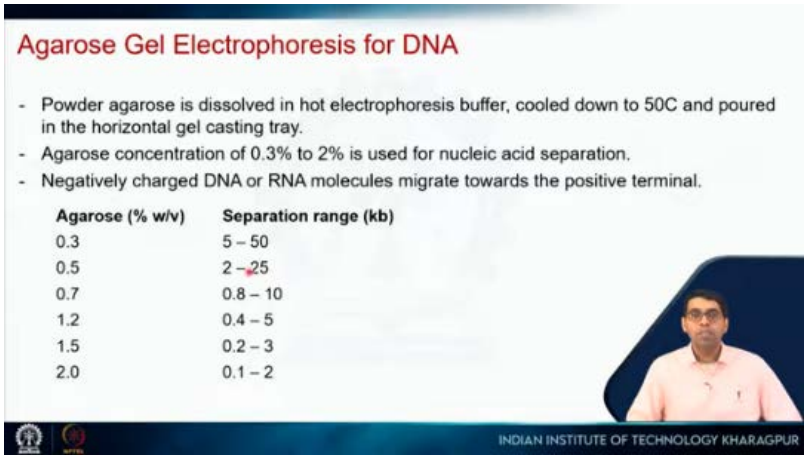


Agarose is the most popular matrix for DNA samples. So, agarose gel is used to characterize DNA and RNA, which are in the range of 200 to 50,000 base pairs. What is

agarose? It is extracted from seaweed and forms a linear polysaccharide. So, this is the typical setup I showed you PAGE, which was vertical, agarose is done horizontally because here your gel is much thicker, so if you put it vertically, then of course there will be compression; the mass is much more, and that will create problems, so the density of your gel will change if you put it vertically.

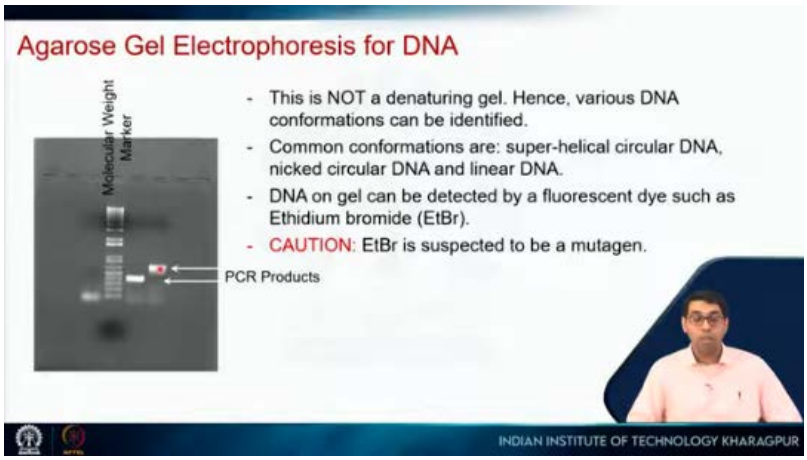


So, we typically run it in a horizontal fashion. Again, this will be the negatively charged terminal, and this will be the positively charged terminal. You can see there are small holes here. So you load your samples here, and then it will migrate from this end to that end because DNA is negatively charged. So, depending on the sample size, you will use different percentages of agarose.

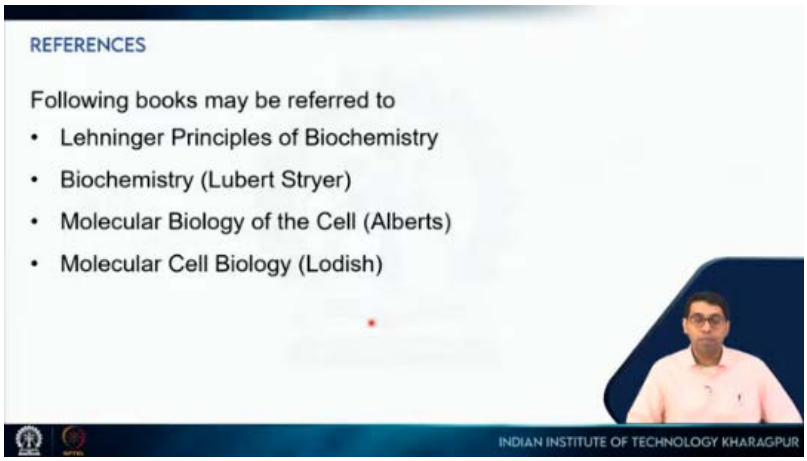


This is the weight-by-volume percentage of your agarose. The experiment is done. Again, you will have to use something to visualize, and typically we use ethidium bromide. Remember that ethidium bromide is a suspected mutagen, so you should be very careful in

handling it and also disposing of the gel where you have stained your samples with ethidium bromide. This is one example shown here: this is a molecular mass marker, just like the protein ladder. This is a DNA ladder, and you have done some polymerase chain reaction and you want to see whether it is there or not. If your PCR has worked, you will see these bands at the correct or expected size.



So, these are the books that you can follow for this particular lecture.



Thank you.