Interactomics: Basics and Applications

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Lecture – 44
Biomolecular interaction analytics using MicroScale Thermophoresis

There are advent of new technologies which are really trying to provide high throughput solution and much more reproducible and reliable results. One way of doing high throughput assays is to tag the proteins of interest with different type of tags or regions like fluorescent tags or radioisotope or chemiluminescent tags and then trying to monitor the signals obtained from them. However, if you are adding a chemical moiety on your proteins of interest it may provide some multi fact results, it may alter the protein binding or the protein functions as well.

So, there is a lot of facets about how to do the protein studies in the label free manner and there is advent of new technologies which are trying to achieve the protein protein interaction, protein other Biomolecular interaction studies by using label free platforms. In this light today we have invited a guest Dr. Sheev who is the head of business operations at nano temper technology to talk to you about a latest technology MST which is Micro Scale Thermophoresis for quantifying the binding events and studying the bio molecular interactions

Dr. Sheev will also provide you a demonstration of how to use MST technologies for bio physical assays. Towards the end one of the colleagues an application scientist will explain the basic principles of Tyco which is used to check the protein quality and purity in a very very short time. So, in this manner you will have a good understanding about how to first touch your proteins of interest, quantify them to check their quantification and also their purity level and then further study how they can bind with another protein of interest or a drug molecule of interest. So, let us have this lecture with Dr. Sheev.

I will quickly start my presentation without wasting much time, but before that I would like to know like are you doing any biophysical assays for the interaction studies protein protein protein small molecule, if you are working on those lines for sure this talk should be very interesting to you. So, I am sure you are working on those lines ok great ok.

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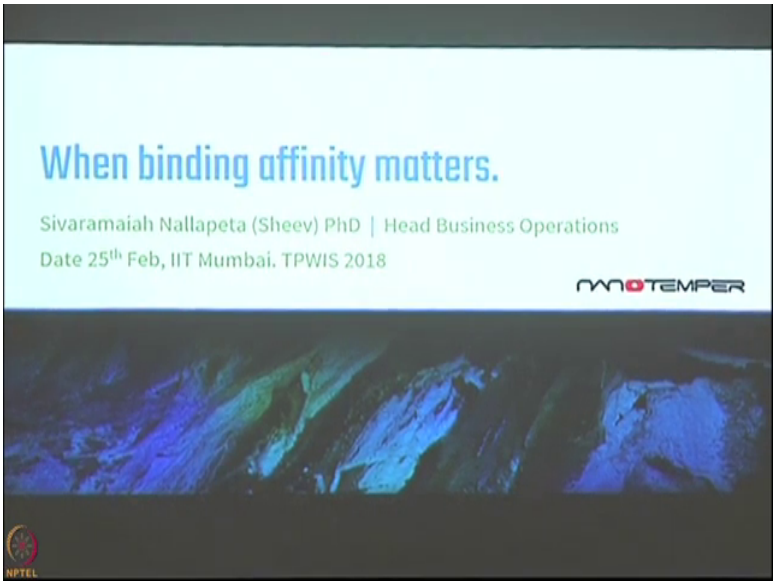

So, this is something like you know a biophysical technique that determines the binding affinities. So, as I said like you know this is a technique about a binding affinities. How you determine the binding affinity between two biomolecules?

It can be anything protein protein, protein small molecule, protein ligand like anything you can determine the binding affinity. So, but before that I would like to quickly introduce myself as myself Sivaramaiah Nallapeta you can call me Sheev, I currently taking care of

nano temper business in India we are based in Bangalore. And my other two colleagues Sanji Menon applicant specialist and my other colleague Ram is also here with us.

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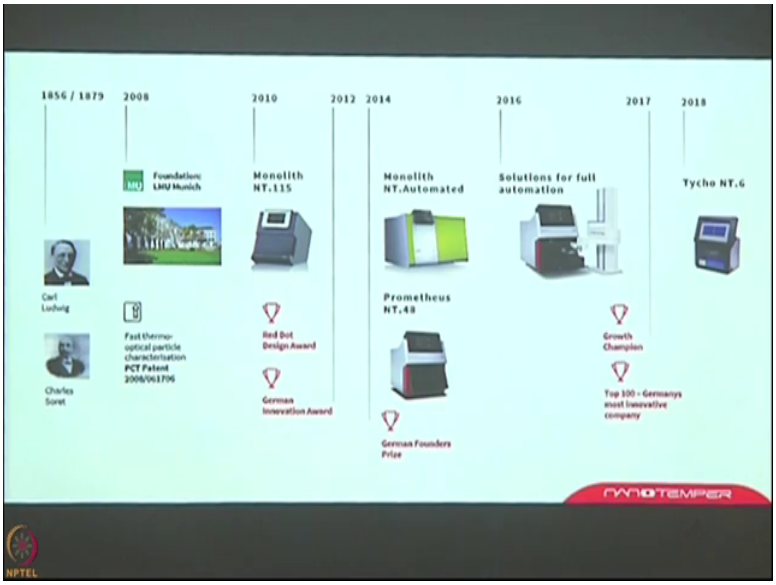

So, before going to the details of the technology, I would like to give a quick introduction and how our company has formed which would be very interesting. The technology what I am talking about is thermophoresis. So, we thermophoresis have you ever heard of it before? Yeah, we say that like you know whenever there is a heat the molecules will move from hotter region to the colder region, but it will be a directed movement. So, this principle was discovered by this two gentleman's 150 years ago, but there was no any application it was only a physical principle, but there was no any application.

Until the two CEOs of our company who filed a patent in 2008, they said that the thermophoresis can be established at a micro scale level which can be used to determine the

biding affinities. So, from there the company has started and as I said like the first instrument which was launched in 2010 which is in front of you, they said that using this thermophoresis principle you can determine the binding affinities. Later on different versions came into the picture which I am really not interested, but other than this we have a one more technology which is the protein ability.

Like you know you just wanted to know till at what temperature your protein is stable like what you call that TM melting temperature of your protein. So, these are the two technologies what company has and as I said like these two instruments are very handy just beside me on that side and this side. Maybe during this talk is over we will also have a quick demonstration and we will see how easy it is to run a experiment without any optimization.

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So, these are the two CEOs of our company as I said like they are 31 year old who has actually filed a patent and started this company in 2010 and now we are successfully spread across the globe. And, as you can see a coffee machine in between them we say that our machines are very simple they are like a very robust like a coffee machine like you know its a vending machine like you want to do an assay everything you just prepare and keep it and 50 minutes of time everything is ready on your table, we will see is it as well.

So, with this quick introduction of the company as I said like you know its started in a small garage in 2008 and we slowly spread across the world and we have more than 700 instruments across the world. And the best part of this company is like you know you see from 2010 to 2017 we got 1700 publications in all reputed generals

So, that is how we had been connecting more to the scientist, its completely at technology driven company and it has been estimated that 15 million experiments are done using our technologies till date. So, I think this is the more than introduction of the company what can I give and now I am just coming into the technology as I said it is a thermophoresis we have established at a micro scale at a very small temperature gradient we have demonstrated this thermophoresis. This is called as the Micro Scale Thermophoresis in a short from you can call it as an MST.

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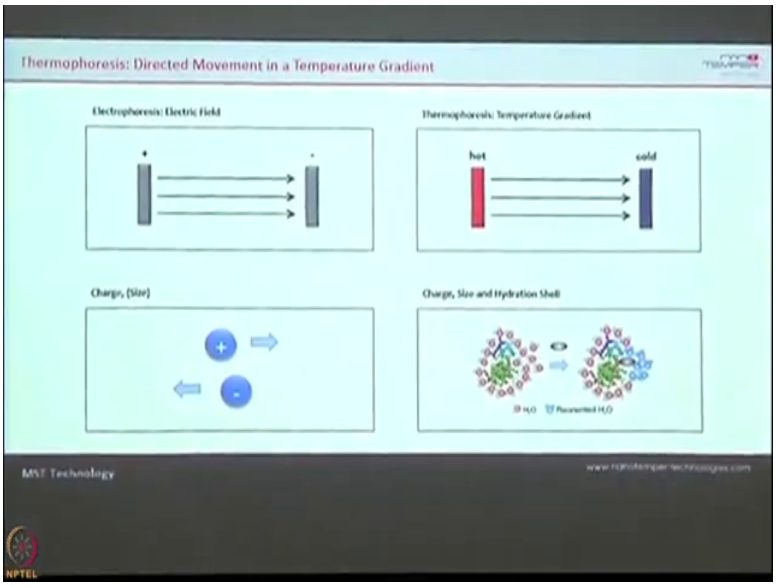

So, but before going into the details I just want to elaborate more on the basic principle what is thermophoresis, we all know what is what is electrophoresis. So, in the temperature in the like in the electric field the ions or molecules will move based on their sizes if there is any gradient in between them like an acrylamide or like any kind of a gel.

So, similarly thermophoresis is the movement of the molecules in the temperature gradient; thermo means temperature phoresis movement. The movement of the molecules in the temperature gradient is called as the thermophoresis, but this thermophoresis is sensitive to for three things; size, charge and the hydration shell. So, as an electrophoresis you are only seeing the size right, but here this movement is dependent on three important things; size, charge and the hydration shell.

So, if any of these things changes during the molecular interaction, the thermophoresis behaviour changes. So, that is a basic principle what we have been using it maybe I will go more with the detail; size, charge and the hydration shell. So, normally when a protein protein binds the size changes, when a protein DNA binds the charge changes and when a protein small molecules binds the charge never changes or size never changes, but still one water molecule is displaced upon the binding. It all depends how it is binding and all these stuff.

So, this technique has become very sensitive for all these three parameters and there will be a change in the thermophoresis behavior when there is any change in these parameters yeah.

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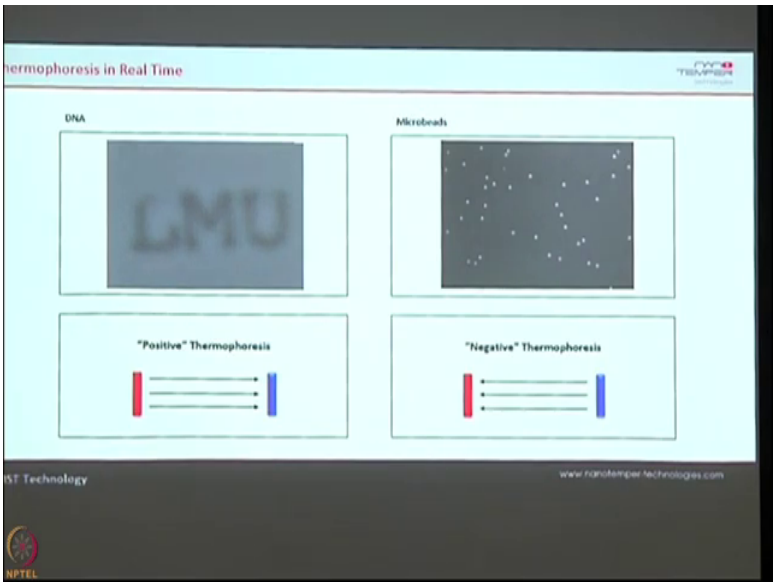

So, generally as I as said like you know thermophoresis the moment you know you put a heat all the molecules move away from the heat that is the general principle. As you can see here this is just a cover slip slide which has been coated with LMU, the moment you are shining

with a laser all the flows and molecules are moving away. So, that is the reason why you are able to see LMU this is the Munich university. So, that is called as a positive thermophoresis, the molecules moving away from the heat which is a general property is called as a positive thermophoresis.

If similarly there will be like you know the vice versa of it what you call it as a negative thermophoresis, sorry. So, in the negative thermophoresis what happens is the molecule move towards the heat. So, it is like example glass beads when you shine some laser or you when you heat them all the molecules move towards the heat that is called as the negative thermophoresis.

So, in principle when you are doing a thermophoresis experiment you always see these two kind of a phenomenon like whether they move towards the heat or they move away from the heat, but in our principle you can measure both their movement; how we measure maybe I can take into the next slide.

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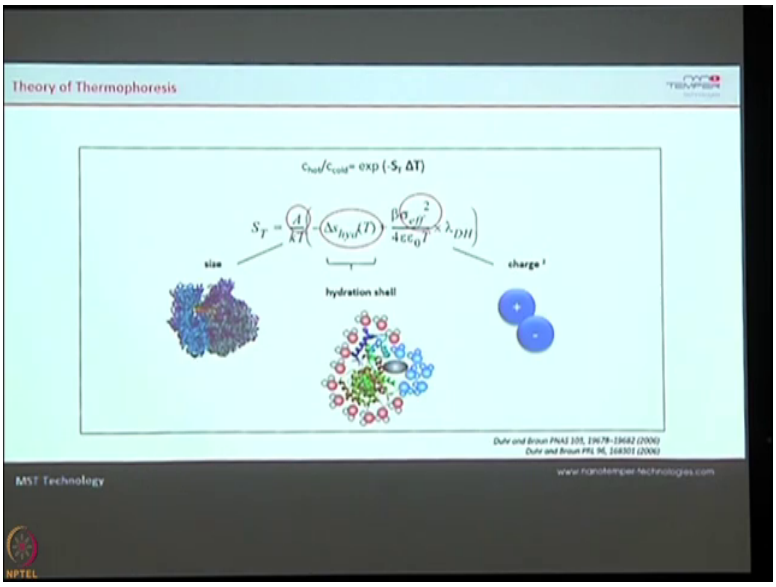

So, if you look into the first slide the two gentleman who has propose the theory of thermophoresis they states that the steady state concertation of the molecules before and heating; before and heating or directly dependent upon the three parameters that is size; charge and the hydration shell.

So, as I said like you know before heating whenever you are shining some heat the movement of the molecules that has gone towards the hotter region and the molecules in the colder region their ratio is always dependent upon these three parameters that is what the law of thermophoresis says. And, all our experiments have been high validated and it follows this principle.

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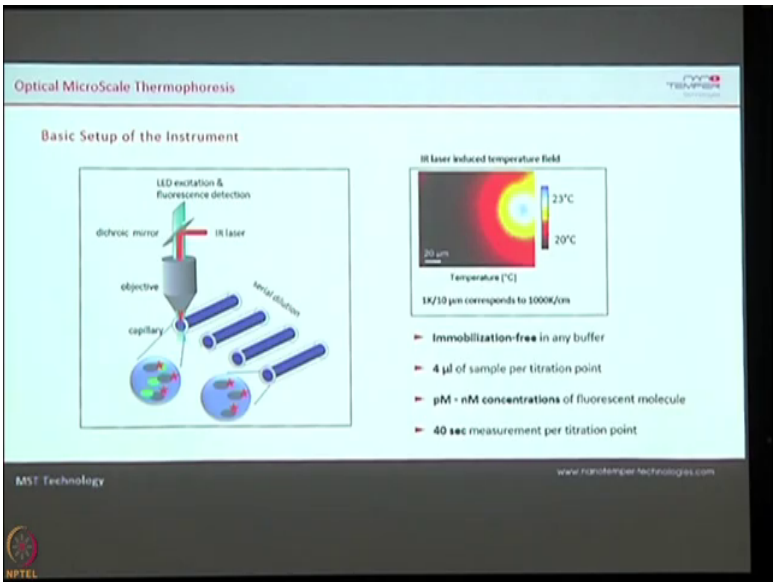

So, with this basic principle of thermophoresis we built up an instrument which has two important things; one is a laser which creates a temperature for thermophoresis and here one of the binding partner is fluorescently labelled. So, just to see the fluorescence you have a fluorescent detection unit. And the biggest advantage in this technique is you are not immobilising anything as in other techniques. So, you directly determine the binding affinity in the buffer.

And you require only 4 to 5 microliters of the sample and the time. So, normally like you do a dilution series up to like 12 to 16 dilutions and every dilution take 40 seconds to scan. So, in 12 to 15 minutes of time you are able to determine the binding affinity without any compatibility with the buffers there is no any cross torque, there is no any like you know a buffer interferences in your assay

There is a biggest advantage of this technique and many people ask the temperature are creating how much is the temperature we create, its a very steep gradient as you can see in the slide 20 to 23 degrees. So, that temperature does not affect any of your molecules. So, in that way what we do is you fix one of your partner and iterate the second one of a different dilutions and irradiate with the laser and you see how the bio molecules move away upon the interaction and when they are not interacting.

So, that differences will help us to determine the binding affinity. So, let us see more in detail in the next slide.

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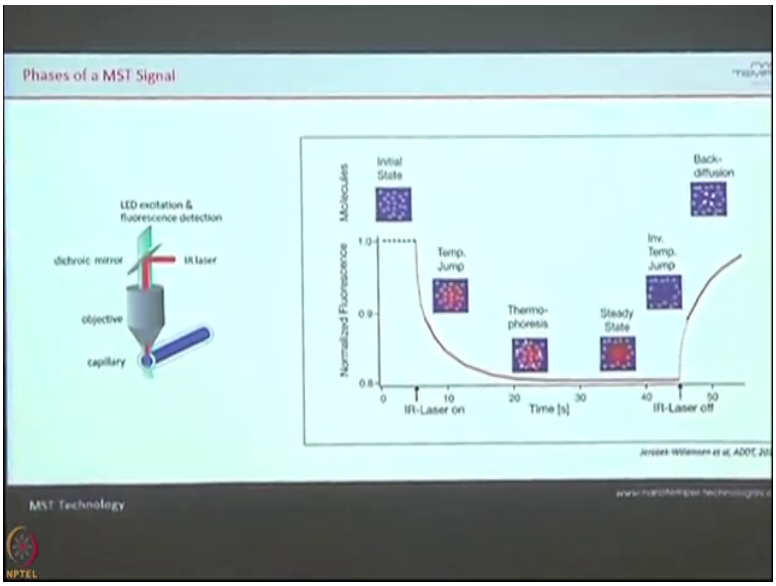

See if you look into the typical signal what is actually happening, as I said like you are fixing one of the partner and the second partner you are titrating it right. So, on every concentration on every dilution whatever you are taking it you are irradiating the laser. So, as you can see

here for the first 5 seconds all the molecules are uniformly distributed this is generally what is in principle it is a, the moment you put the laser on IR laser on what is happening is all the molecules are moving away from the heat.

And it states that within 30 seconds they attain the steady state. So, for 30 seconds I am putting the laser on ok. So, what is happening is all the molecules are moving away from the heat and within 30 seconds they attain the steady state as you can see here and after that I am putting the laser off since there is a fluorophore again there will be a like you know regaining of the fluorescence. So, gain your signal will shoot up right, fine.

So, now I have a sample where one of the binding partner and different concentration of a ligand is mixed and that has been taken a small glass capillary and on every glass capillary I am taking this thermophoresis reading. First 5 seconds it is very uniformly distributed after 5 seconds you are putting the laser; till 30 seconds we assume that it attains the steady state and after that you are putting the laser off.

So, in this way for all the different dilutions whatever I am taking it I will collect the thermophoresis signal right.

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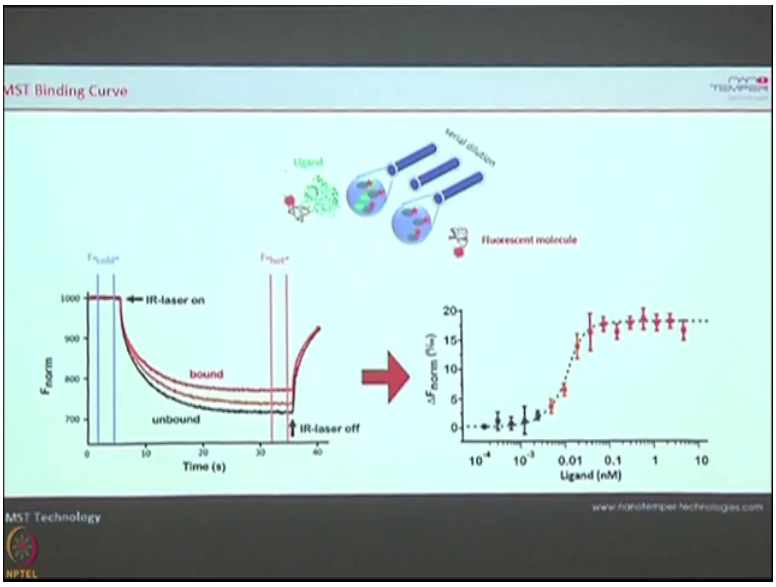

So, this is how typically as I said like you know in the top you can see and each and every glass capillary I have a partner along with a ligand which is like you know a different concentration. So, now, every of the glass capillary is going for the thermophoresis measurement you see here. So, there is a change in the movement. Can you tell why this change is happening?

Student: (Refer Time: 13:38).

Yeah upon binding either the size or a charge is changing. So, that why the movement is also changing. If the movement is not changing; that means, that there is no any binding at all then you have a signals all are overlapping one over other as a clutter, but here as you see here the

as and how there is a binding the different concertation of the ligand is coming the movement is changing right.

So, this is how we track the difference between the unbound state and bound state, the unbound is where it is not binding and the bound state is a where the biomolecule is actually interacting. So, in this way we are actually capturing these small differences in the thermophoretic behaviour to determine the binding affinity.

So, once you determine all the 16 thermophoretic curves for all the different ligand concentrations you just go to the dose fit curve as you can see here this is the base line where typically there is no binding. And here this is the saturation and the midpoint is generally as a K d is what we call it as a binding affinity. So, where your molecules are half bound and half unbound is called your K d.

So, typically by just extracting the data from the thermophoresis curve we are fitting the dose fit curve to get a K d value. So, this is the basic principle what it is underlined in the instrument just by the thermophoretic behaviour changes from the bound state to the unbound state you are able to determine the K d.

Since this technique is very sensitive for size, charge and the hydration shell you always get a differences in the thermophoretic behaviour which will help you to determine the binding affinity. The biggest advantage is you are taking a smaller volumes and you have no interferences with the buffer and there is no any limitation with the sizes.

So, this is the basic principle I am sure like you know if you have any questions you can stop me here the rest are like you know how the examples like you know how protein protein interacts, I have some good data I want to show it to you. So, this is the basic principle how this instrument works and how we determine the binding affinities.

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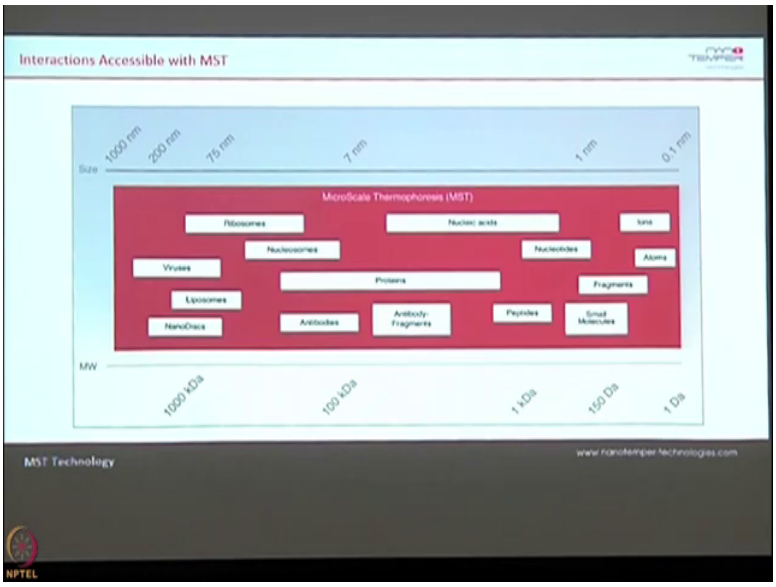

So, with this you see a wide variety of samples of from ionic interaction to the liposome interactions, nano disk any kind of interactions can be studied in this instrument. So, there is no any limitation of size here, there is no any limitation of any of the properties of the molecule here.

So, anything even like a ionic interaction you can imagine the ions are almost in a Daltons and even if you know liposomes or ribosomal complexes they are in a mega Daltons, even if there is an interaction between the these three things still the techniques works very nicely. As I said like you know we got 1700 publications in the last 5 years we have got all the supporting data with all different kind of interactions we have.

So, if you are working on a specific like protein protein, protein small molecule you can still discuss I can help you out like you know how we can do the assay another stuff. So, this is the

main USP of this technique without any limitation of the sizes you can work with the wide variety of the samples to get the binding affinity.

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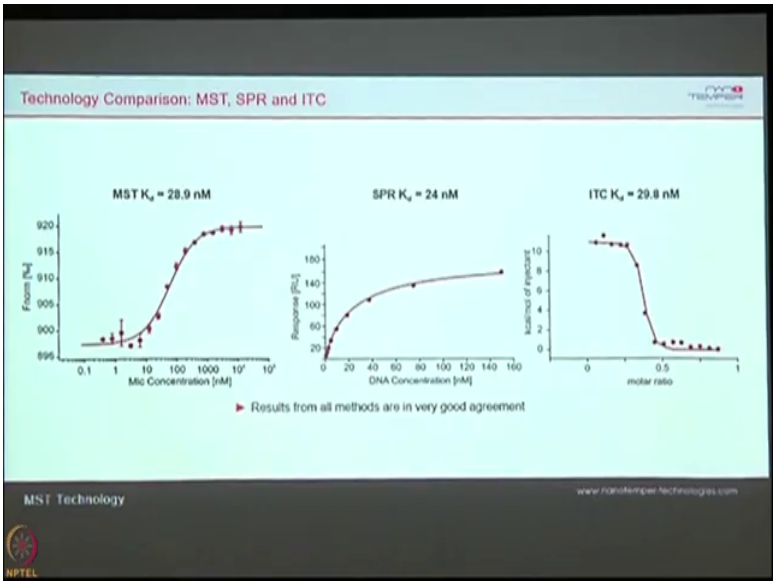

So, typically this technique is comparable to the SPR, have you ever heard of SPR I think many people might be using the SPR as well.

So, this technique is a very much comparable to the SPR and ITC Isothermal Calorimetry. So, in the chemistry people will be just measuring the heat change, heat exchange to determine the binding affinity. So, the biggest advantage over all these techniques is as I said like you know we did a protein DNA interactions and you see in all the three techniques you are almost getting the same K d, we got 29 nano molar, SPR 24 and ITC be got 30 nano molar.

So, whatever the binding affinity you determine in the MST is very similar to the ITC and SPR assays.

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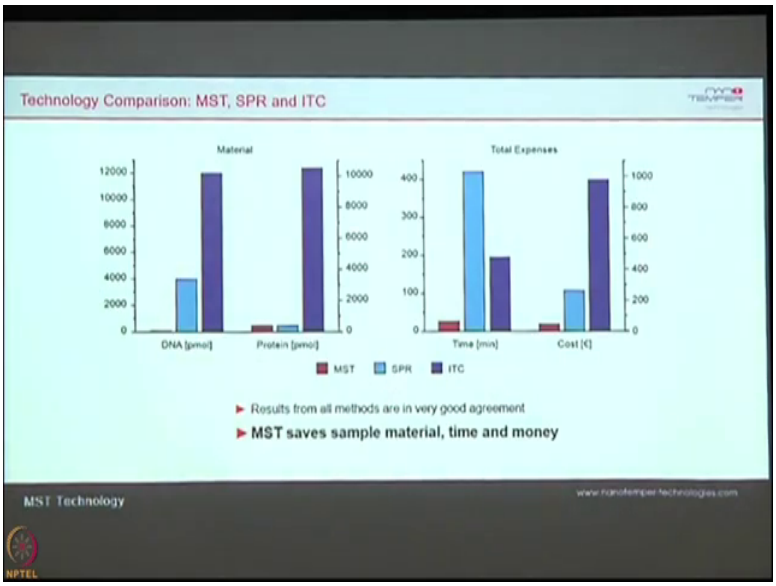

But what is the biggest advantage? So, as we are all familiar with the SPR you always have a chip you immobilize one of your protein and pump your different buffers and ligands and it involves a lot of method development and method optimization and of course, the cost for every assay. And in the ITC we know like you know you have a huge pump and then you need to fill your pump and then start putting it up.

So, they are higher volumes of the sample is required. So, if you look into the biggest advantage of the MST is you do not require the higher volume of the sample we look with the always the lower concertation of the sample and the assay is done in 10 minutes of time with

no cost. The glass capillaries are only the consumables what we use for doing the assay and it will cost you 5 Euros per assay.

So, at the end of the assay if you look on like to perform 1 K d measurement you require 5 Euros. So, that is the biggest advantage the smaller volumes, time and the overall cost of the experiment. So, this is been a highly like you know worked out with all the three experiments as you can see here the cost you know its almost like one tenth is what we generally do it for a SPR another assay experiments. So, many people working with SPR still may have some questions you can still feel free to ask me.

So, as I said like you know we do a labelling to another binding partner. So, we recently came up with a kit where you can label your His tag.

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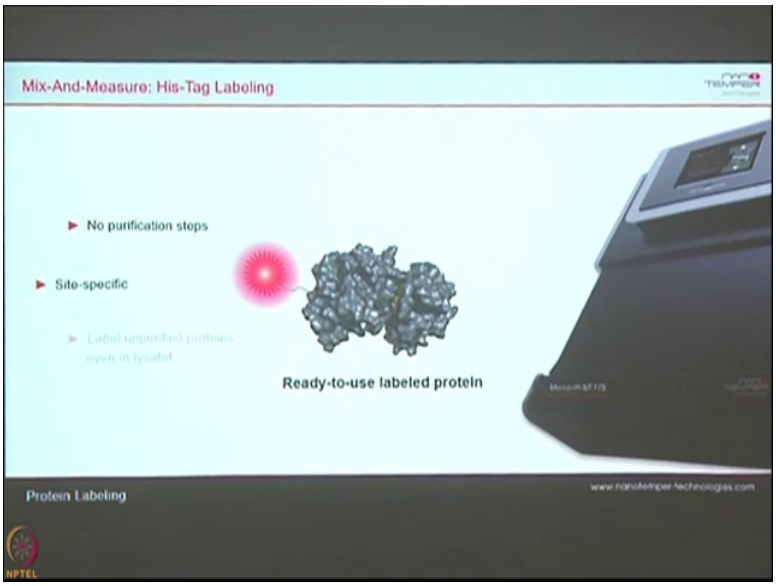

So, at the 6th histidine position you can label your protein. So, in such a way what is the biggest advantage is like you know you need not purify your protein, if your protein is in the cell lysate and it is already His tagged just simply at the di and do the ditrations. So, in that way even in the cell lysates if you want to have a specific interaction studies this can be successfully done.

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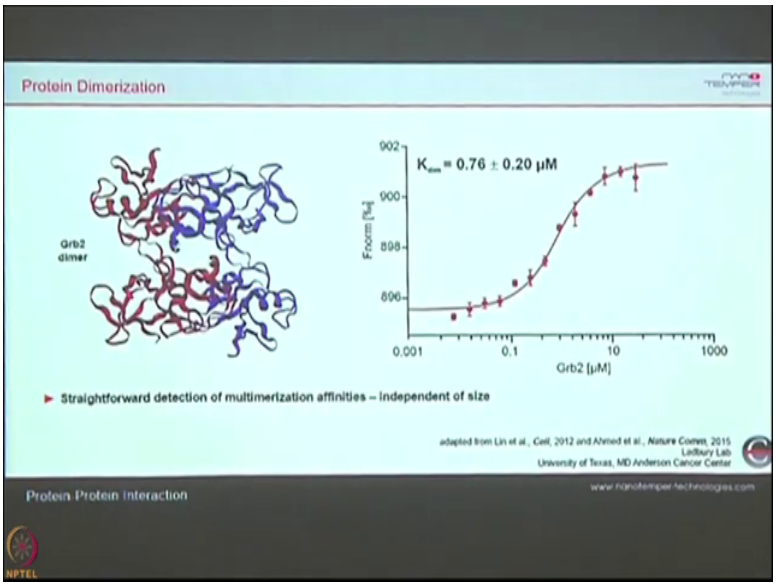

So, now I will just take into the some examples of like you know how this application has been used in the different variety of interactions; one is the dimerization. So, when you are talking about the protein protein interaction the same protein when it interacts it becomes a dimer.

So, its extremely difficult in other techniques like maybe in SPR or in ITC when the same protein you are emoblycing and same protein you are running through you get a lot of

artifacts. So, but here labelling one of the protein and titrate in the same protein you can determined the dimerization K d as well. As you can see here you clearly see a clean fit of K d coming up here. So, even the protein dimerizations and protein dimers can be easily studied using this technology.

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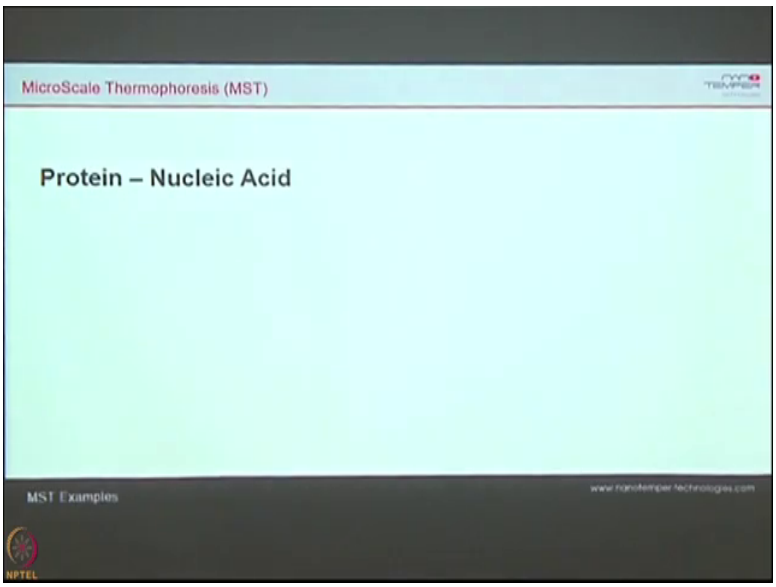

And other most interactions people are interested is protein nucleic acids.

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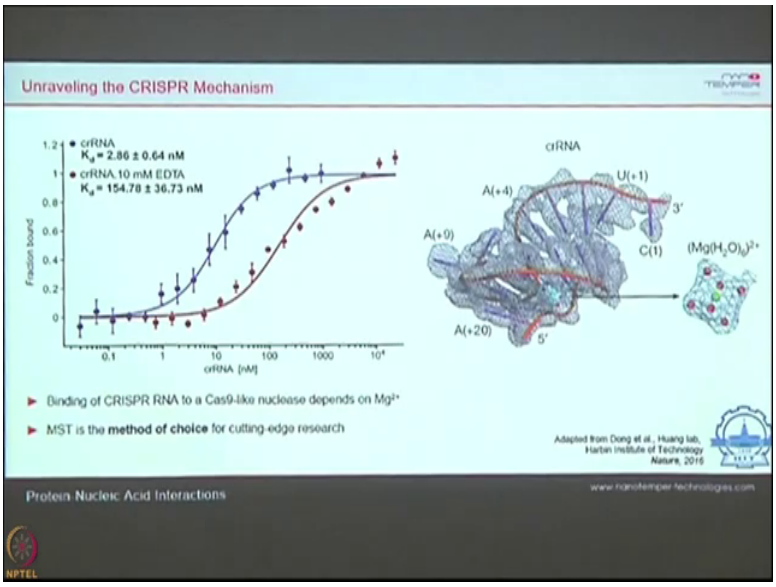

So, I am sure you are well aware of this CRISPR mechanism. So, here are the RNA to the nucleus protein has been studied and this paper hit the nature and here in this paper they quoted that this is the only biophysical technique that has been to determine the binding affinities.

So, even the cutting edge technology is also MST has become much handy tool to determine the binding affinity.

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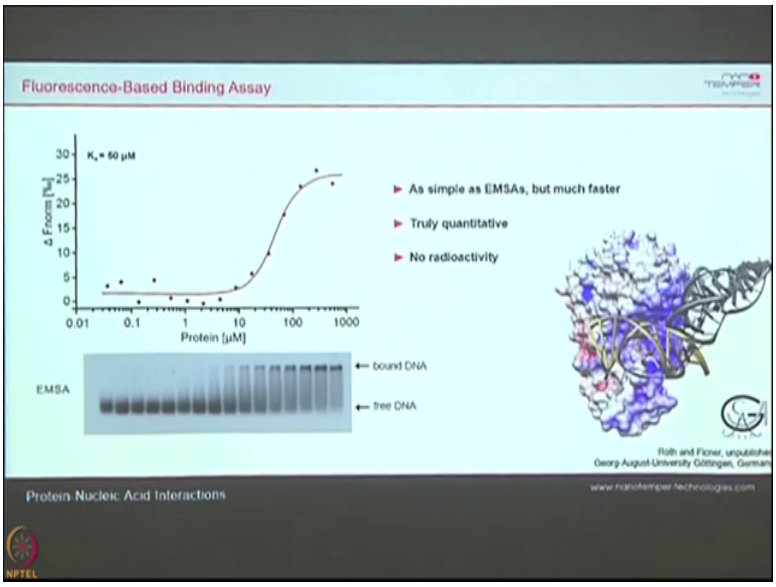

So, maybe traditionally people does EMSA. So, have anybody done EMSA here oh great. So, you know like what is the pain of doing an EMSA there and you always get qualitative and you never get a quantitative information there. So, in the same studies whichever done with the EMSA we try to label the DNA like a psi 5 or psi 6, so whatever you have the dye.

And then titrate your other things and you are able to determine the K d which is very much similar. So, you are with the smaller volume of the sample you are quantitatively determining the K d over this all these EMSA and radioactive labelling everything is all been like you know over looked when you do this kind of assays here. So, other example is a protein ionic contractions these are always a very special interactions as I said like you know when a protein and ions interacts there is no size at all, there is no any size or making any difference

The ion is a very small in size and your protein is a very big. So, that time the size does not change at all, but still when a protein goes and binds there is a displacement of one water molecule at least outside the protein or anything. So, even with this small change we are able to determine the binding affinity this is the biggest advantage as you can see this is a one calcium binding protein which has been done. And when the same experiment was done in the presence of a magnesium there was no binding as a positive and negative control this experiment has been done.

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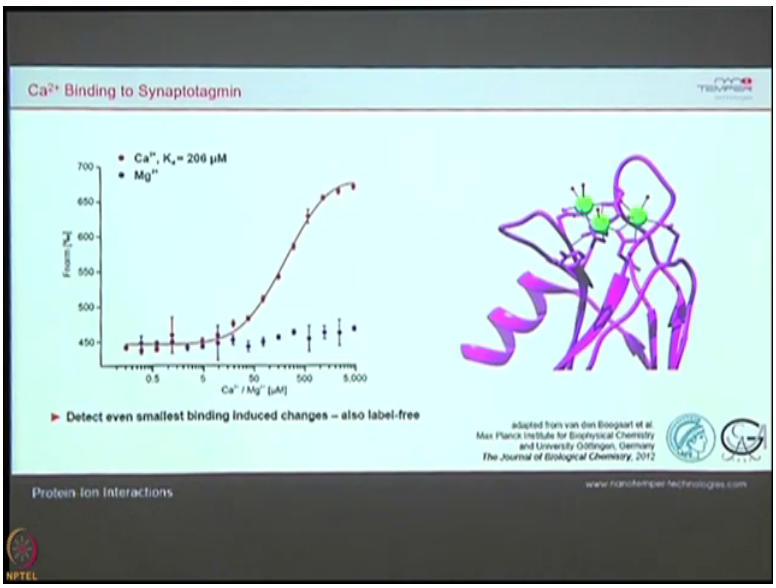

And, as you can see here this is from the Max Planck institute the person was has developed the ITC who is like you know known to be the father of ITC. So, always when you are working with the nanomolar, picomolar interactions and a very small size molecules this addresses very nicely over any other techniques.

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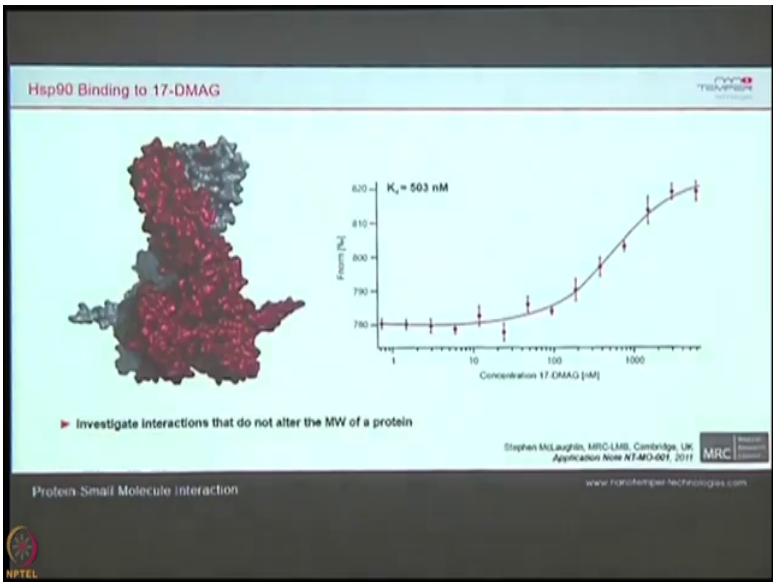

So, again this is like you know a protein small molecule interaction here you have a ribosomal protein complex in the red color which is like you know few mega Daltons. And a small molecule which is about like you know 120 Daltons the cancer drug. So, when it binds the size is absolutely not like you know making any difference like if you imagine like mega Dalton protein and a 120 Dalton small molecule interacts the size does not change at all.

But, in principle if you look into the structural information like you know the water molecule is actually being displaced which we call as the hydration shell. Because of which we are determining the thermophoresis change and the binding affinity.

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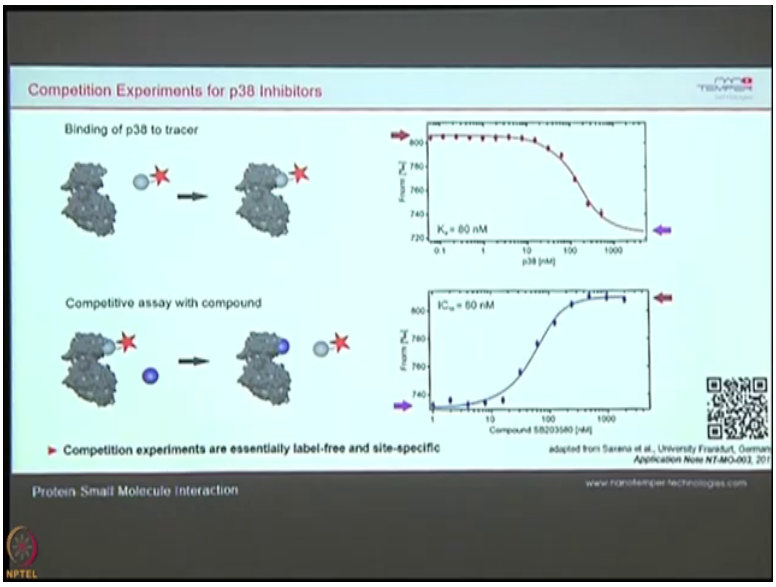

So, an other experiments many people who are working with the enzymes they might be more interested with the like this kind of what we call that as a inhibitor studies inhibitor screenings. So, generally as you know like you know you do a binding with one specific what you call this the tracer molecule and once you know the K d of it and again you titrate the second one.

So, wherever it displays it then always know the binding like competitive assay experiments can also be easily studied. We have a well robust software to support all these kind of studies at the end of the day.

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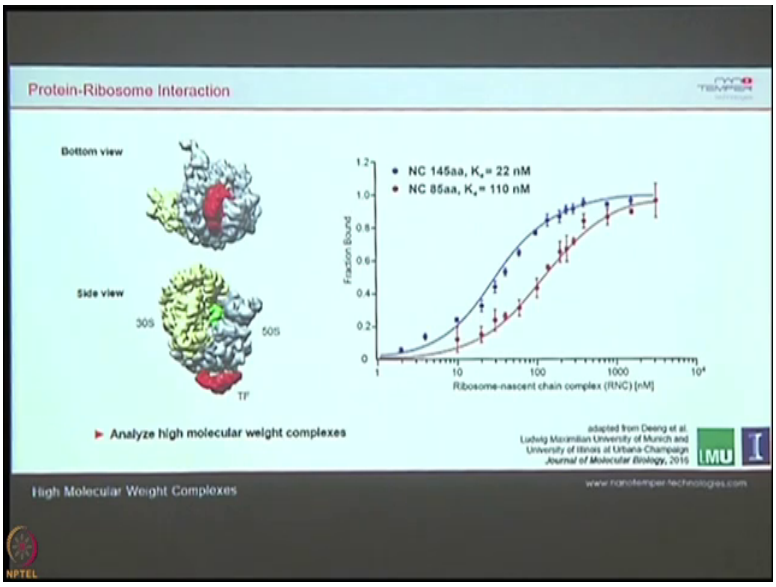

So, this is again a same example with the high molecular weight protein complexes as I said like you know few mega Dalton proteins when interact with the proteins still you are able to determine the binding affinities very easily.

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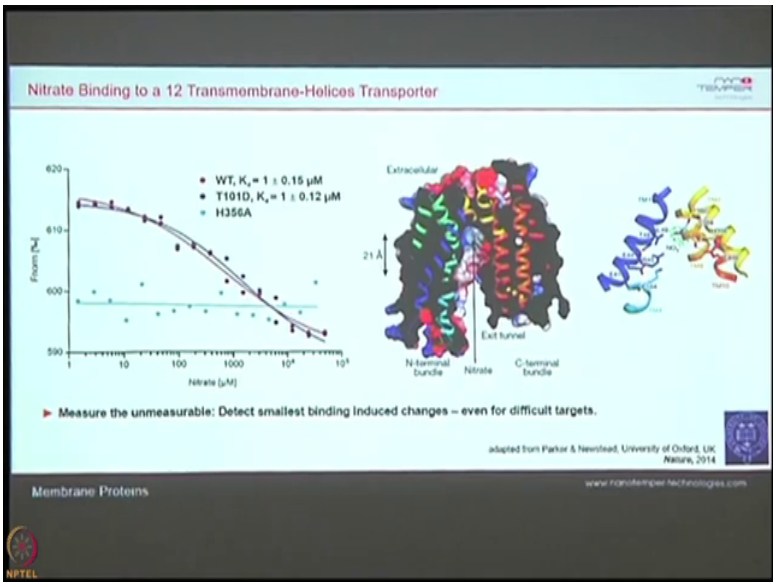

And, the other challenging thing is like if anybody is working on the membrane proteins hope you know what is the pain of like making the membrane protein more stable, it requires a detergents. So, any other techniques like you know when you have a detergents you always have an a buffer incompatibility for suppose if I have an STS if I have some any like a foaming agency in your things, then; obviously, you get lot of artifacts.

So, here in this technique as I said like we are using only 3 to 4 microlites of the sample that thing can be nullified. So, even membrane protein can suceessibly be studied here as you can see paper again hit the nature where the membrane bound protein has been express with the GFP and that particular protein has been studied for the micro scale thermophoresis.

So, in this way any variety of sample it need not be a protein protein any kind of molecular interactions, protein carbohydrate, protein ion anything in between them can be studied using the micro scale thermophoresis.

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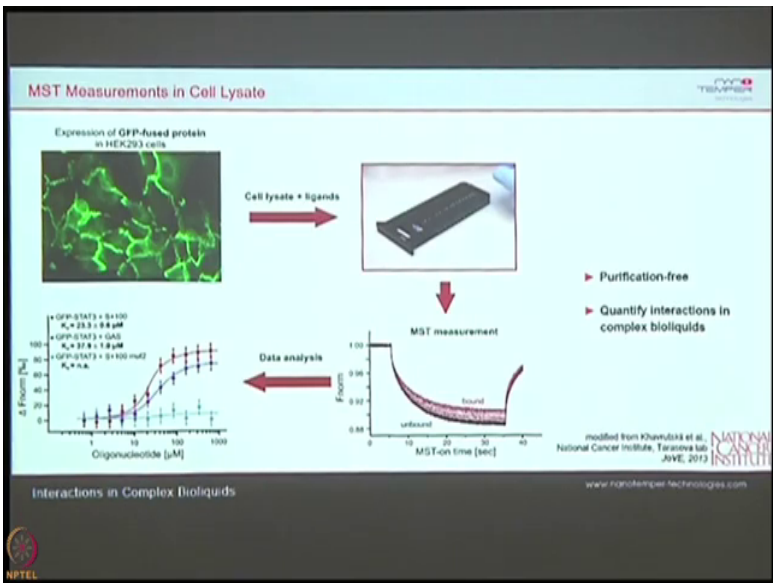

As I said this is one example where the cell lysate like when you are working with the biological fluids like maybe a serum, cell lysates you have a thousands of protein. So, you can even bypass by doing them a not doing any purification. If your protein of interest is always with the His tag you add the dye which is very specifically binds to the His tag and then titrate the other partner.

So, in this way even for diseased markers many people working with the proteomics are also using the MST as a complementary tool to screen the different samples for the analysis here.

So, this is again just the examples like how we get the data here. So, as I said like you know any one of the binding partner should be labeled, it can be A or it can be B.

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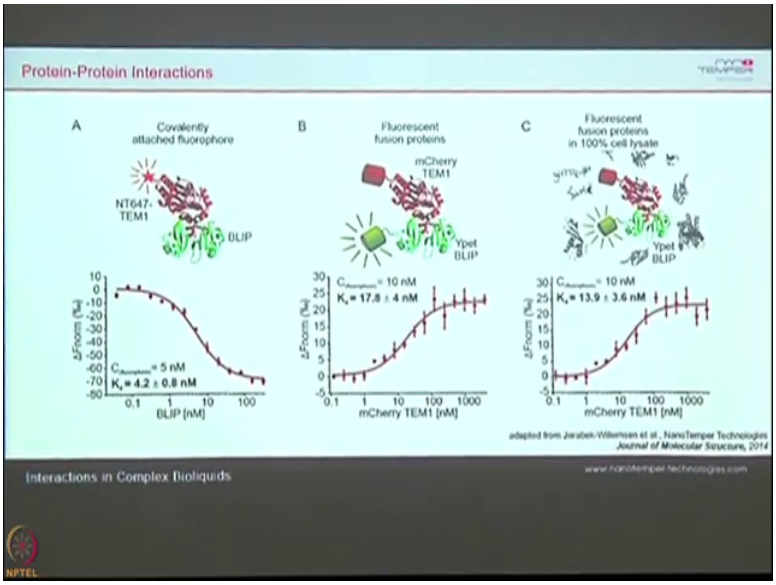

There is no mandate that you know you should label only a protein you can label anything. You can label a small molecule, you can label a protein any partner can be labelled, but the binding affinity does not change at all.

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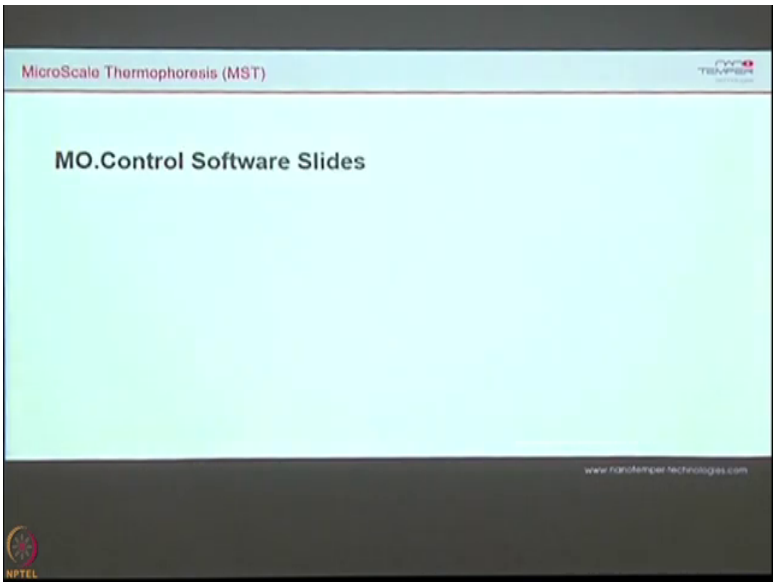

So, that is a main USP of this. So, these are some examples what I want to tell for a variety of interactions how these techniques works so successfully. So, any questions or any other things you wanted to me know I shall be glad to address. So, now, just as I said you are aware of the principle how we does it and you know what variety of applications can be done on those. Now, I will just quickly take to the software like you know how this instrument like how it can be operated and all these things. May be a in a nutshell I just want to tell this software is an artificial intelligence driven.

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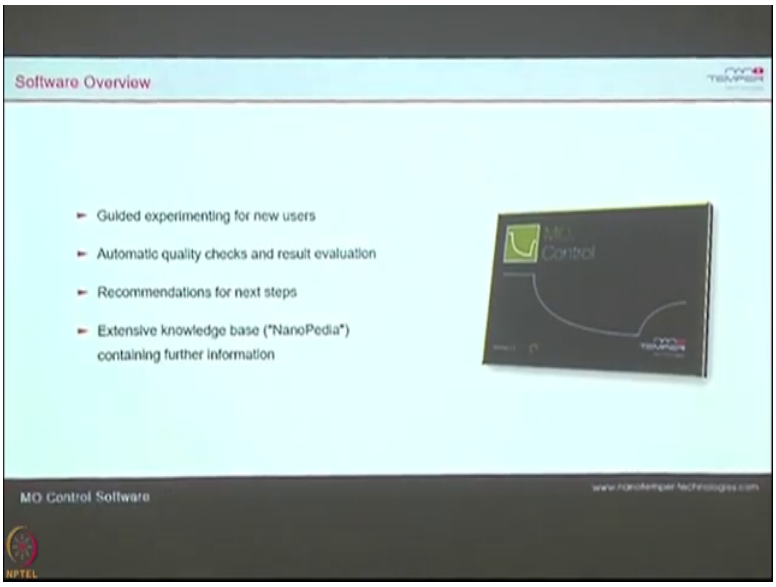

So; that means, that the moment you setup the assay it runs on its own.

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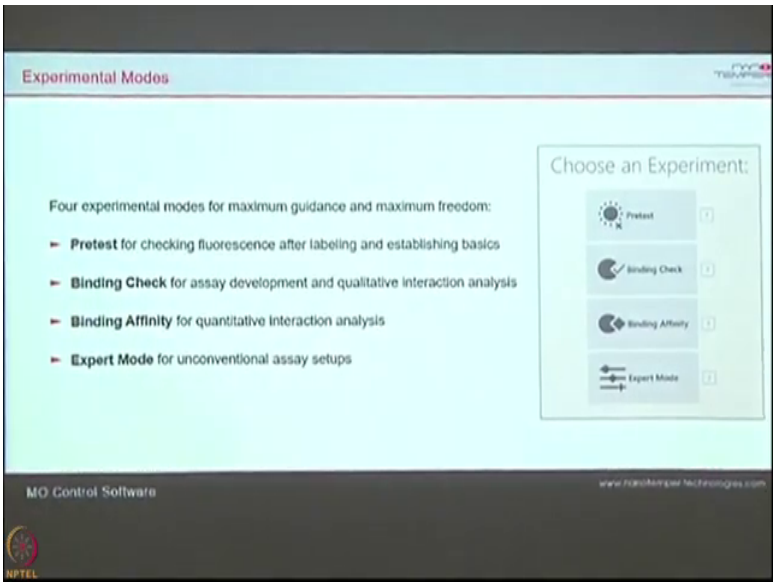

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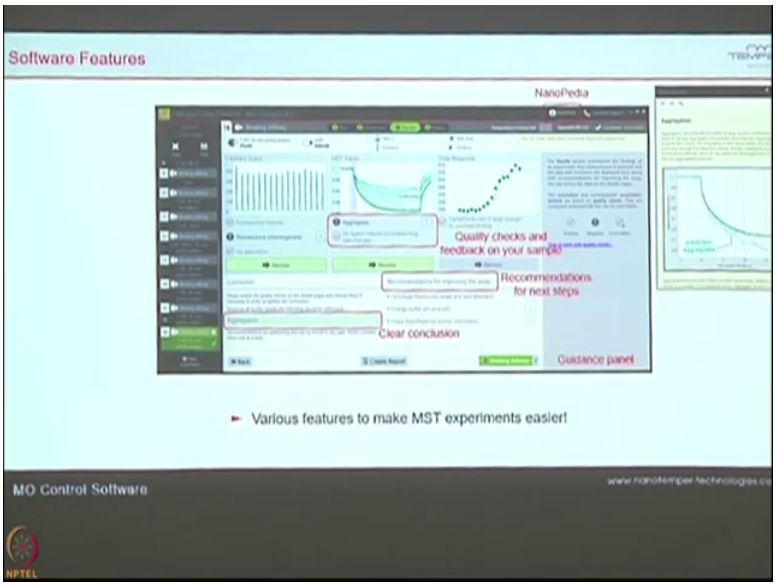

And once the analysis is over the software automatically assesses whether this data is good enough or not is a something like you know still you are not getting a proper results out because of any reasons it will automatically give you a suggestions ok. If this is not a binding fit then it will tell you please change the buffer you change the pH or you change your like you know the protein concentrations

So, like this automatically software does the assessment. So, in a way it is very much easier of course, like you know as a user we always know technically what is happening with the software by looking to the data we can always assess, but still software will help you to make a judgement on your analysis and you always get all the data in a single page as you see here this is the clean binding fit.

So, and these are all the thermophoretic tracers of all your dilutions what you have made it. So, for suppose like you know your samples are making an aggregates. So, for suppose like you know upon at particular concertation where it is interacting with the ligand, it becomes an aggregates. So, then aggregates means then the sample will be nonhomogeneous, the thermophoresis behavior also will be very like random the curve likes you know looks very very random I will just show how it will looks like.

See sorry, see whenever there is an aggregation see you see here the curve is completely like you know a wavy. So, like this qualitatively we can always judge the aggregation of your sample also. So, in other experiments when a sample is aggregating it is already in contact with the instrument its spoils the next one, its spoils the next things. So, the biggest advantage here is you are using a different independent capillaries for every dilution.

So, if one capillary is having an aggregate you can just discard them and you know at what concentration your protein is actually aggregating. So, in this way qualitatively you can judge the aggregation in your samples by using this kind of a technique.

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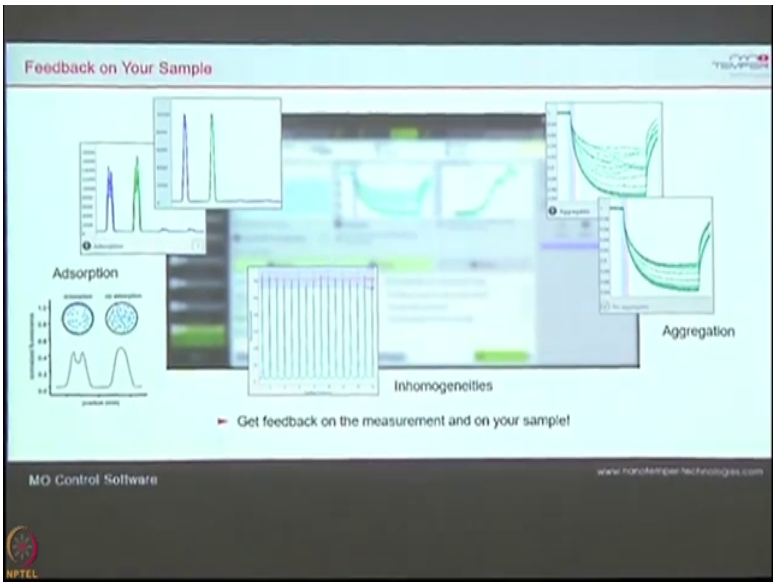

So, maybe this all these things I do not want to bore you much because as I said like its more for a users who are actually doing it. In principle the software is completely a artificial intelligence driven, it gives you a suggestions at the end of the day like how we need to move on.

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So, this is the just I just want to summarize quickly we are already associated with the top premium institutes across the globe and I am very glad that in India we are associated with the IIT Hyderabad, RCB Novozymes and many other customers who are using this technology.

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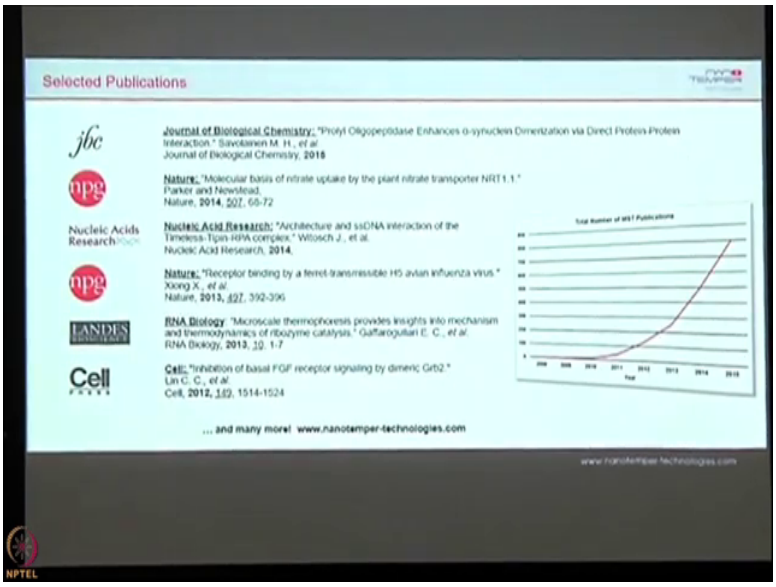

And as I said like you know in all kind of generals you can find the MST papers like in in nature we have more than 15 papers science are all the best journals have acknowledged this as the biophysical tool that actually be a very useful tool in measuring the binding affinities.

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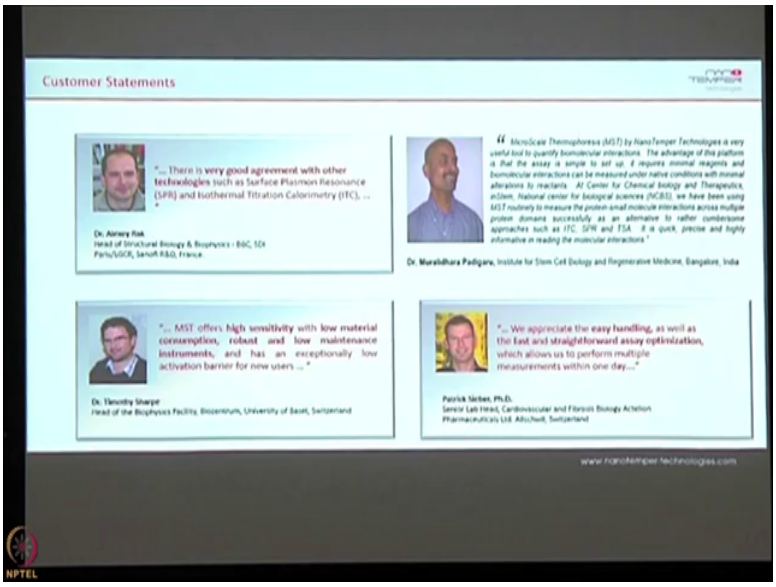

So, here are some customers who had been using a lot of SPR, ITC experiments and now made a shift to the techniques and found how easy it is, like if you are doing any screening like you know you are doing a drug screening you have a small molecules libraries hundreds or thousands of molecules. So, I am sure like you know other techniques will be a night mare. So, here since you are using a 10 to 12 minutes of time you can always screen them very quickly.

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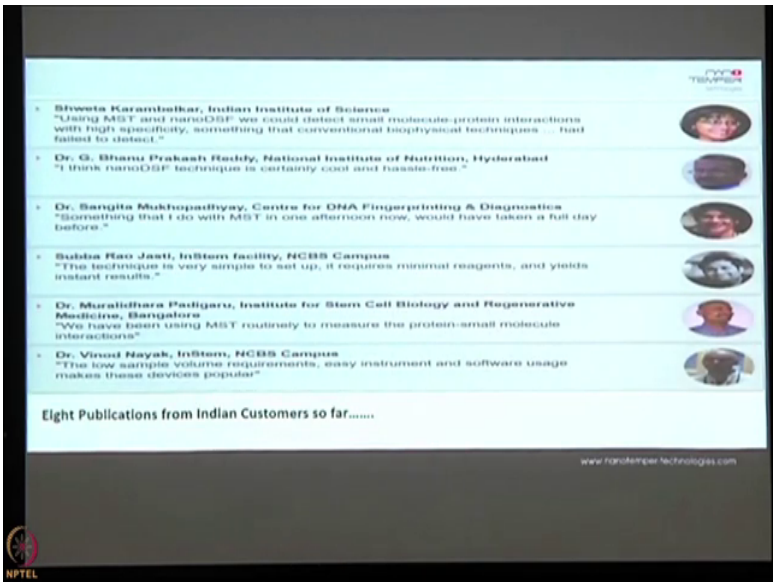

And, these are our some customers who has used it and gave a very good feedback and we have more than 10 publications from Indian customers in the last 24 months.

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The final slide is always what I say as I said in the beginning it is a company of scientist. So, along with a its not just telling about the instrument it is all about giving a good support. So, you are always bundled with a good scientist to support you and develop the assays on your own.

So, that is all I want to tell since we already or going to give a quick demo maybe like you know we can just quickly share some questions you have or if you are working on a other things what are the challenges you are facing, I shall be very glad to help you out. What kind of samples you do on a SPR basically at the moment?

Student: We are trying to do ITC.

ITC ok. So, for sure like you know as I said like now we have a small a positive control kit it is a DNA aptamer and a small molecule ATP basically ADP and the DNA aptamer. So, anybody is interested they can just come in front and then just do it because as I said like it does not require any much training much thing to understand. As I said the basic principle is thermophoresis and how the every dilution there is a difference is what we need to understand and the rest everything is done by the software automatically.

Student: Protein and small molecule interactions or protein and this alkaloid interaction.

See that is what I am saying in principle anything is possible alkaloid can you tell me an example.

Student: (Refer Time: 30:57) curcumin.

Curcumin. So, very recently I think as I said like 15 minutes of the time is a measurement time. So, maybe people can come front and this wanted or you can sit there and see; however, your wish. So, we have a control where we are just going to load into the capillaries and do the like an MST assay and we will go to this one the stability one. So, there also the things are ready it takes only 3 minutes to measure the stability of your protein that is the biggest advantage.

So, maybe she will give you a small talk on few slides what is the basic principle and what are the applications. So, anybody working with the protein can use this instrument for quickly checking like you know the functionality of your protein whether your protein is really functional or not ok. So, these are the glass capillaries what we use for the assay, you can see this are the simple glass capillaries which can accommodate maximum of 5 to 6 micro liters, you just dip into the solution.

So, normally you take a PCR strips and then like you know fix your partner a and titrate the ligand like then only like 12 to 16 dilutions is what we prepare and all the 12 to 16 dilutions each dilution you take in the glass capillary. So, and this glass capillary will go for the

measurement. So, and here you have already like you know we already mixed the DNA aptamer and a ATP here.

So, what I am just going to do is now you know I am just dipping the glass capillary and then loading it. So, its all like once you load it within 10 minutes of time you can do it. So, normally you know proteins are very like you know unstable at a room temperature. So, the DNA has been used the DNA is very stable here. So, for the assay. So, the generally for demos I prefer to do with a DNA because its more stable you can carry anyway what you wanted.

Yeah. So, this is a tray which holds your glass capillaries. So, its like labeled 1 to 16. So, like whatever you are taking it you just try to put into this and then do it and we have a small magnetic strip to arrest your glass capillaries its like you know when you are loading it they might not fell down. So, its just a magnetic strip to arrest the glass capillaries when you are loading it that is all. So, maybe it will take 2 more minutes to load and after this we can just start the assay yeah.

Yeah yes somebody want to load they can come and load, you can load it no problem

Student: (Refer Time: 33:12).

Or a protein purification by columns or anything like that. So, how you maintain the quality, how you check the quality that is nothing, but the stability or the functionality. So, we have recently launched this instrument only not even a 1 month old is this. So, we can quickly check the protein quality how what is the basic principle of this and what kind of a applications, she will try to summarize and give it in just 15 minutes of time.

And after that that instrument also we can give a demo it takes in principle only 3 minutes to run one like run 6 samples, we got some controls we can quickly run that also. So, Suchi please go ahead.

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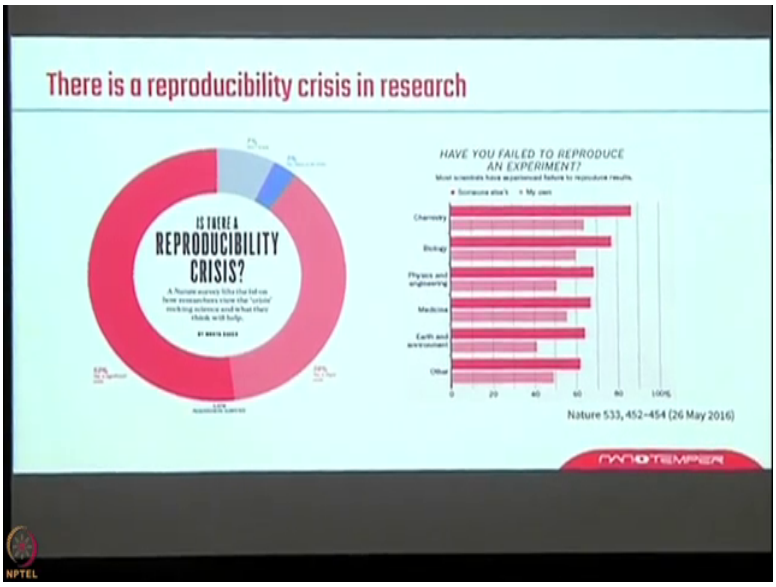

So, have you ever asked to yourself that whatever a protein you are purifying whether its pure or not, I mean whether that can be used for your future assay experiments or not. So, that is a very big question that always comes in the mind of any researcher. So, before going for any assay what you do is like you run a column you do a STS page or you do spectrophotometric analysis. So, these are like very standard gold methods going for a spectrophotometry or electrophoresis or a column chromatography.

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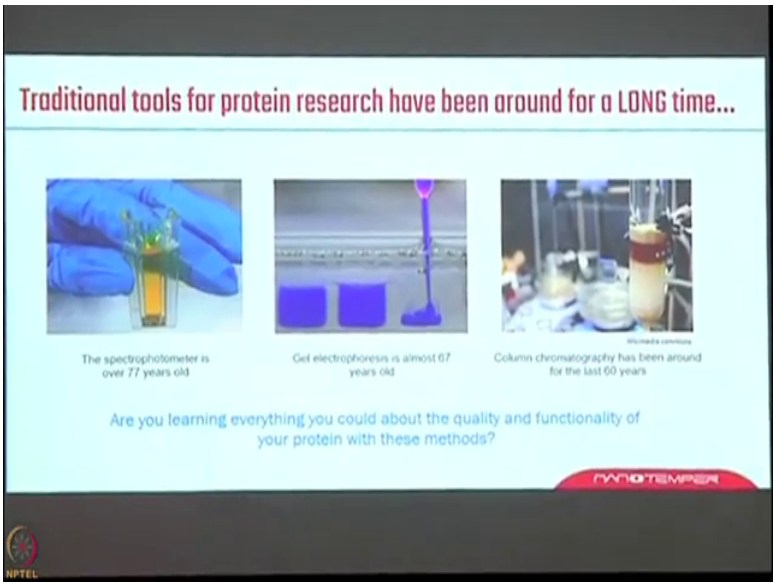

But still you are not aware whether your protein is a functional protein or not.

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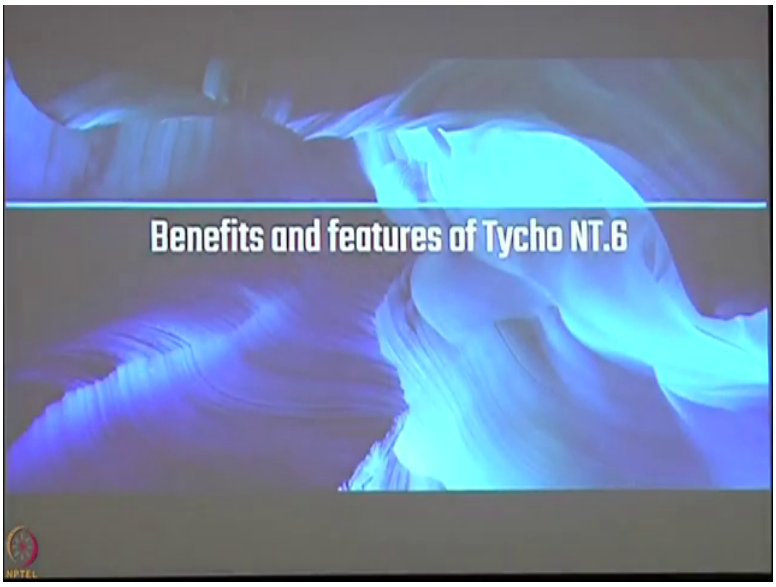

So, we have this newly launched product which is known as Tyco and in one go it takes 6 samples and you can actually check the quality of the protein. So, it will come to know whether the protein what you have you know purified is functional or not.

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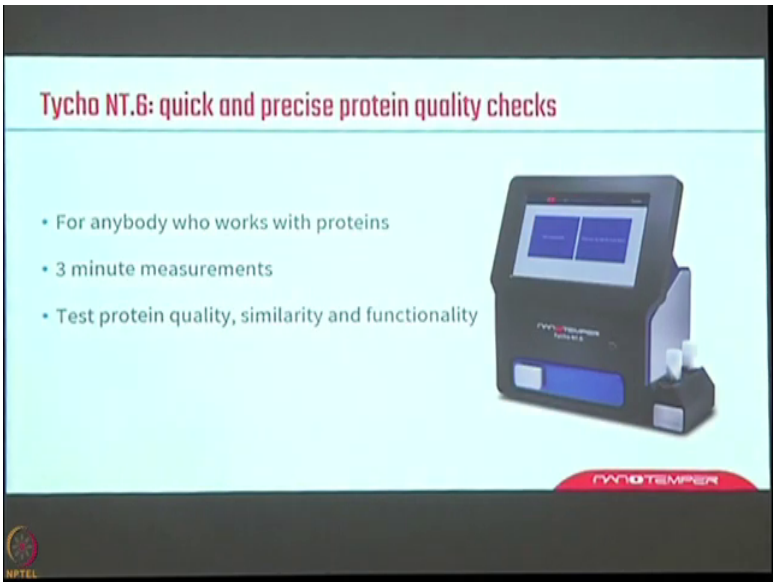

So, what is the basic principle of this machine is that it can work with any kind of protein whether its a antibody or whether its a normal protein membrane protein receptor protein any kind of protein it give a 3 minute measurement So, within 3 minutes you are actually checking your quality and the purity of the protein.

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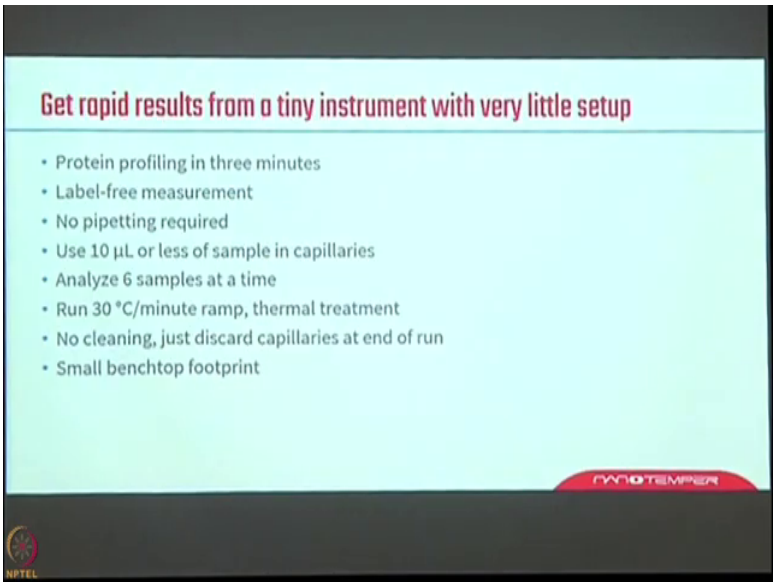

So, its a label free measurement like here one of the partner is labeled you are not labeling here is the intrinsic protein fluorescence that we are checking which will give you a label free what you call inflection temperature which is very equivalent to the melting temperature. And you are similarly using capillaries and the amount of volume the volume that you are using is less than 10 micro litre and in one go almost 6 samples you can analyze.

Then the run rate here is little fast because you are analyzing you know in three minutes. So, the run rate here is 30 degree per minute and as of our all the instruments are maintenance free there is no cleaning you are just putting the capillaries in the instrument taking of once the run is complete. So, what are the basic benefits here is that its the faster measurement technique and it checks the purification and the I mean it checks at the purification and the characterization levels.

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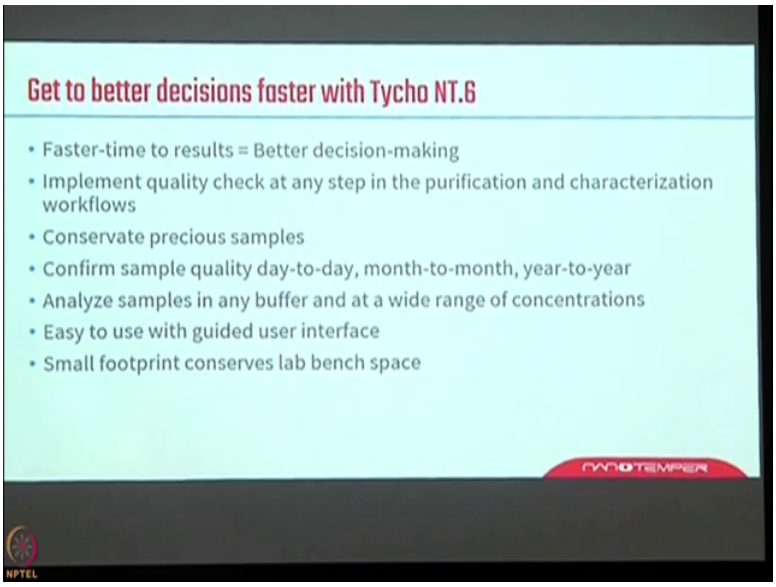

So, you if you have like batch to batch samples or like 1 day old samples or 10 day old samples or like very old samples and you are freeze thawing the samples all that samples when you take and check one by one. You can check the actual purity whether it has denatured or not like that you can always check batch wise batch wise. Then you can analyze the samples in a wide range that is from 0.5 microgram per ml to 250 mg per ml and as you can see its a very small instrument you can hand carry the instrument here and there.

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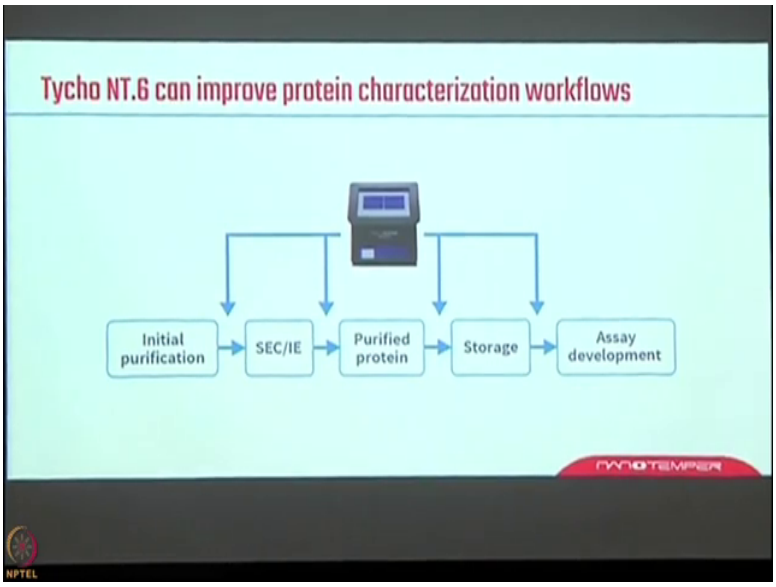

So, its a very ease to you know work with. So, when you see the characterization workflow where all the Tyco can be used. So, when you go for the initial purification after that when you get the protein you can check the purity at this level after chromatography I mean ion exchange chromatography a column purification you can again check the purity of your protein. Once the protein is purified, then you can again you have to store it at different conditions. So, at which buffer, at which pH you want to store it you can always check that and then store it in that conditions

Once you have stored the proteins suppose you have kept it for 10 days or 20 days after that you want to again check the purity because for assay you need to have a pure protein if its not pure then definitely let us suppose you are going for SPR, one chip you cannot waste if your protein is not pure. So, before the assay development you always have to check the protein

how the protein is, if its totally denatured and you are doing an assay definitely you are you losing your time as well as the chip what you are using for a SPR.

So, all these are the steps where you can actually check your protein quality. Now, I have few applications where it will tell you where all Tyco can be used.

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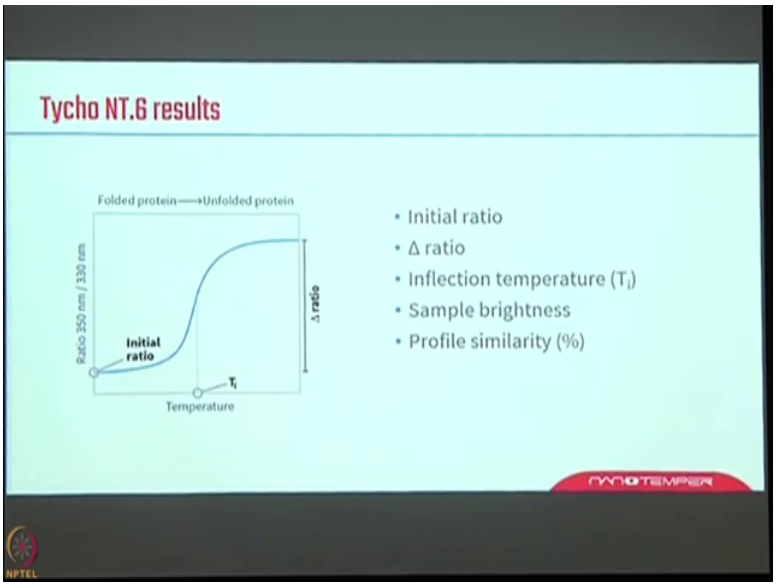

Now, in this when you see what is the basic principle that is applied here is that it is checking the intrinsic tryptophan fluorescence. So, protein from a folded to a unfolded state it will give you a particular T i value. So, the temperature the results that a Tyco is going to give is the initial ratio; initial ratio is the ratio where you know it will give you the purity of the protein I mean whether its denatured or not. And the delta ratio is the ratio which is from the folded to the unfolded region like that when the protein folds when the protein unfolds you will get a T i value as well as the sample brightness.

So, sample brightness here will give you a relative concentration of your protein molecule, it will not give you a actual concentration, but a relative concertation that yeah this much amount of protein is present. And, then protein profile similarity is that if you have a reference sample of your protein that can be taken as a standard protein sample and then when you are going for batch to batch or like if you are doing a freeze thawing at that time you can always take that as a reference sample and cross check the other batch to wise or you can freeze third ones and then check how much it has denatured or how much it has changed.

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Now, here is one example where you have compared it with batch to batch you know screenings. So, here you can see the protein which is a pure protein and a protein that has been kept in minus 80, it almost showing the similar profile, but when it is used after one week, but kept at 4 degree centigrade the profile has changed. So, you can see the shift has

gone up; that means, the protein has slightly unfolded. So, that mean you can definitely go for assay, but still you can check the quality of the protein how it has varied.

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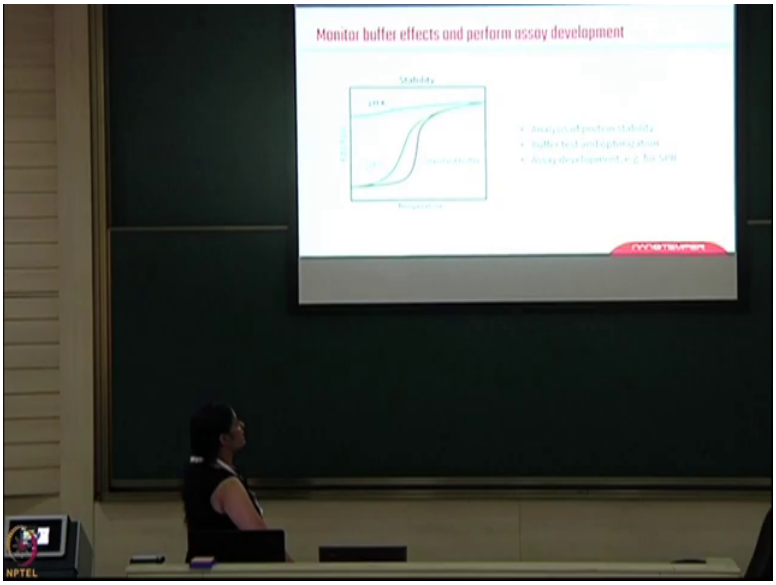

Now, here you can check the buffer staining as well as the pH stability measurements. So, you know like basically for SPR you need low PH. So, if you have if you can if you have a standardize form of how to do it you can check at pH 4, its you know its totally denatured and then at pH 5 which is like a basic pH for SPR measurements the curve is almost good. And when you compare it with different buffers you you can always check which pH you can use which buffer you can use.

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Now, here you can also check the thermal shift assay I mean a protein is there and if you want to check whether the protein can bind with the particular ligand or not. So, when you singly when you run the protein without the ligand you will see particular profile and then with the in the presence of a ligand the shift will change, the shift can be either be a positive shift or a negative shift.

If it is a positive shift; that means, its a true binder, if its a negative shift; that means, its not a true binder. We can actually go for a yes or no that it whether the shift is happening or not I mean whether the interaction is possible or not. With this you can go for further assays like if you are going for SPR or if you are going for ITC you can say that oh this protein with this ligand its good because there is a thermal shift because the TIS are shifting here

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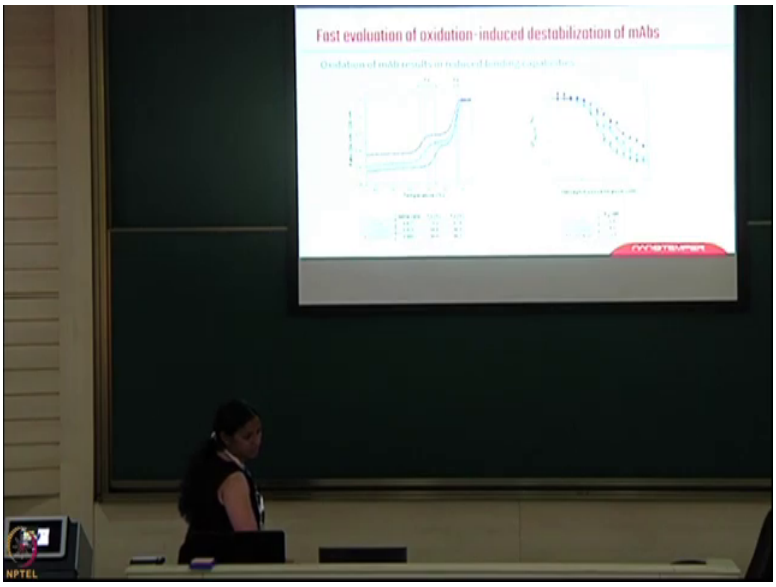

I have few examples which will show you the data you know this is example in which the monoclonal antibodies was checked at different oxidation levels. So, you can see at the native state it has given a different profile and then 3 hour oxidation with hydrogen peroxide it has given a different profile and a 18 hour oxidation its giving a different profile. So, you can see there is a TI shift in all the 3 samples and when you had gone for the MST assay the same thing you have you can see, the shift is I mean the K d value is changing with respect to oxidation.

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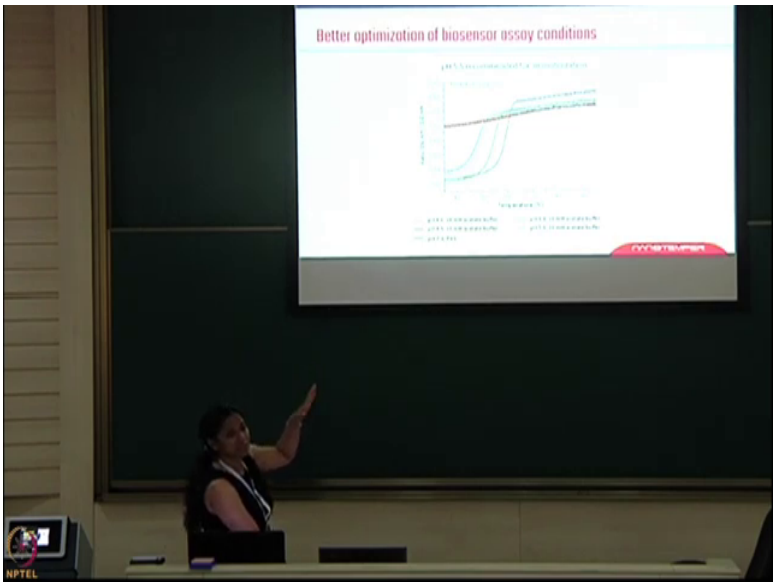

If you want to go for a biosensor assay like the SPR. So, here you have to check the pH values definitely which pH is best for the SPR. So, you can always screen different pH ranges and check which buffer is best. So, like here kinase is taken as a standard and at different pH its tested. So, you can see at pH 7 and pH 4 and pH 4.5, its just giving a planer line; that means, its already denatured ok. But the other in pH 7 and pH 5.5, its giving a curve which is showing the stability of the protein.

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You know this is a thermal shift assay that we have done where we have taken a kinase and then the kinase is treated with a ligand molecule, you can see the shift in the assay you can see protein alone was giving a shift at 50 55 and once its added with the ligand the shift has gone to 62. So, that is how you can go check the thermal assays.

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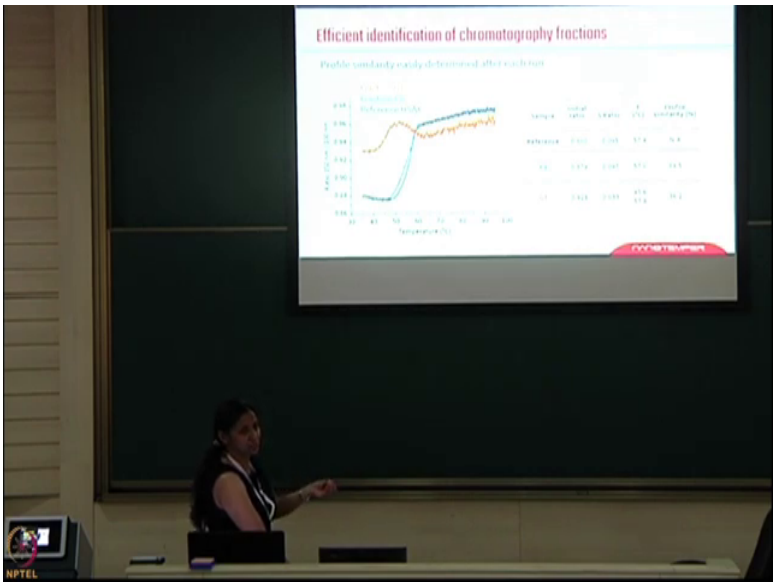

Now, this is once the chromatography is run, I mean you get lot of fragments. So, you check all the fragments and see which is your best which is the best fragment where your protein is there.

So, this will give you a protein profile that this fragment or the first fragment or the second fragment is having the best protein, but if you have a standard reference along with it you can always validate that the protein is actually the pure protein.

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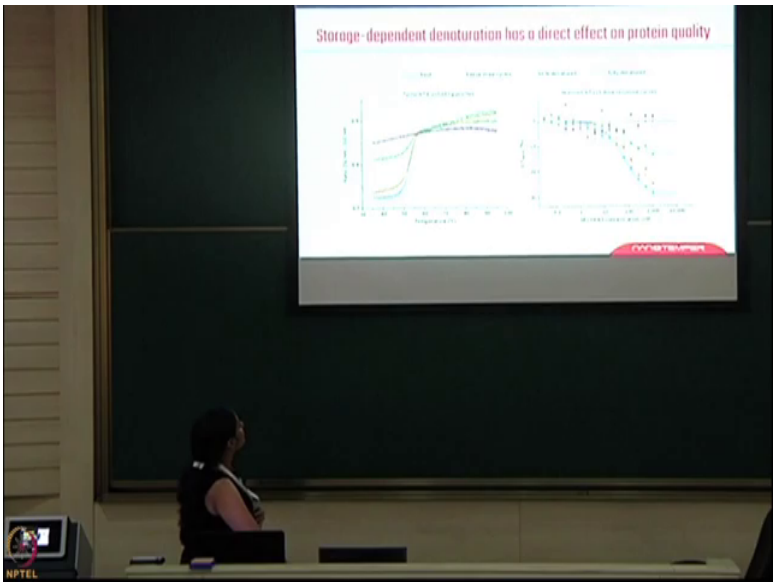

You know here is another example that we have conducted where you have taken fresh samples freeze thaw cycle samples and fully denatured and partially denatured samples. So, you can see how the trend has changed when you are checking the unfolding profiles.

So, the fully denatured sample is giving you a very planer profile whereas, the partially denatured is giving a different profile compared to the freeze thaw and the fresh samples. So, the same is analyzed with the MST. So, you can see the fully denatured sample is you know there is no K d value to it, but the other samples you can get a K d value, but the K d value will vary compared to the fresh sample.

(Refer Slide Time: 45:33)

So, in all if you see its a very quick tool to determine the purity as well as the you know stability and functionality of the protein and when you want to characterize some kinases some receptors or some membrane protein or transmembrane proteins like that you can always check the purity of those proteins. And like it it will give you a yes or no signal when you want to do a thermal stability assays or you want to go for SPR assay or a cryo EM assay or nomas studies

So, before that you want to check whether your protein is stable or not whether your protein is pure or not. So, yes or no will always this this machine will always help you with the yes or no decisions. So, I can give you a small demo with the one sample that is the BSA there is 1 mg per ml I have taken. So, I can just give a its you just 3 minute run that will be there and we can check the profile how it goes.

So, anyone?

Student: I have a question.

Ok.

Student: Do you want any sample of this long term reference proteins whether they change sustain?

Yes, I.

Student: (Refer Time: 46:43).

Student: yeah. So, you have tested many sample proteins reference proteins that can be (Refer Time: 46:53).

As that reference proteins we do not have, but in.

Student: (Refer Time: 46:58).

Yeah.

Student: All those things.

But like if we do for you.

Student: (Refer Time: 47:02).

Yes. So, like if we have a reference protein we we can always you know if you give us a fresh sample, if we have a standard protein we can always correlate with that.

Like you know we say positive shift is goes a right side and the negative shift is goes to left side. So, left side is always destabilizing your complex (Refer Time: 47:20) protein.

Student: (Refer Time: 47:22) we start suppose to give.

Yeah that is in principle know.

In principle now you are saying

(Refer Time: 47:28) storage conditions or some any deposits.

Because in few of the demos we have seen in this kind of shift where there is a positive shift as well as a negative shift. So, positive shift.

Did you get your questions? Sorry.

Yeah, yeah, yeah. So, the positive shift will always say that yes its a binder, but the negative shift is destabilizing your protein and you can see the profile when its goes above 0.9 or something whether it is fully denatured or not.

Student: When we store a protein being denatured as days pass on, it will be somewhat it will be unfolded also it will not be in a stable condition throughout the (Refer Time: 48:06) basically the protein structure is like that only it is not in a (Refer Time: 48:10)

That is what I know your.

Student: Yeah.

Your statement is very (Refer Time: 48:13) like I cannot say yes I cannot say no (Refer Time: 48:15) can depends upon your specific protein what you are working.

Student: Yeah again when we taken when we do the assay assay process we will not get the same results somewhat it will be downloading the result come back to original.

Exactly that is main purpose.

As long as (Refer Time: 48:30) then it is fine if your proteins unfolded with 30 40 percent then; obviously, like you know you are working with the protein which is a negative sign like suppose you are immobilizing the protein and you are changing lot many purpose and you trying to fill everything and if it not working the problem is your protein is really not in a like you know in good state actually.

A quick check point; obviously, taken care of and when you are doing a purification like you know all the fractions you need not go to the STSP and other check and return. So, quickly you can always check it and you are storing it and you are going for next assay. So, this basically you are connecting check point to know what is the quality of the protein what you are working with and today till now you do not have any direct method to like you know measure this kind of a quality (Refer Time: 49:15).

You do the quantification for spectometry black fold other things and you do their column purification, you run the STSP for this many check point you know that the protein you are working is very still.

Pure or not.

Functional or not. So, so when you work with the bio assays unless your protein is not functioning is not pointing that you know setting a assay and spending lot of time and since

its like you know a label free do not require anything like you know you just dip it and then do the machine. So, that would be the quickest way to check the protein quality.

And many times when you take the proteins out.

Student: Yeah ma'am.

I mean you keep it in nice, but still you taking in out in out. So, you know whether the protein is stable or not. So, this is like a quick tool.

(Refer Time: 49:58) she also show that percentage unfolding like you know as I said like you know after 10 days after 1 month.

Student: Yeah.

What is the percentage of unfolding? Say as well as 20 30 percent is fine when it goes above 50 percent, then show like you know (Refer Time: 50:10) everything changes, then that is not your true protein what you are actually working on.

So, in background we never know what is actually happening it when you are taking from minus 20 or minus 80 you are thawing it your protein might be spoiled, but you are not sure, but still you are going for the bio assays. You does not work then you think that you know there is somebody else from, but you does not know the protein itself has being like you know unfolded while doing this thawing or (Refer Time: 50:33).

Student: And one more problem is that here in this we are taking at 30 degree centigrade at per 1 minute. So, at that temperature protein will not get denatured.

See our basic principle here is see when you see a protein the tryptophans are actually buried inside right (Refer Time: 50:52) the tryptophans are buried inside the protein. As a longer heating down the tryptophans are gets exposed once the tryptophans are exposed the analysis

is same thing. So, when completely all the tryptophans are exposed your protein is lost its like you know the activity or like you know the complete unfolding a ceremony.

So, that is the basic principle what we are taking in and we have run the 30 minutes per minute from 30 to 90, we are reaching the 3 minutes quickly because just you are heating the sample and once the all the tryptophans are exposed in your sample then you are getting a like you know what you call that transition like TR temperature point.

So, that is the basic principle, but we have other version of the instrument where you can get 2 TMS melting temperature like you know 1 degree per minute or like 2 degree per minute and you can always control the quickly and you can get the TM values also it is very similar to DSE. But this one is something like for any bio assay any mass spectrometer like you know you are working with intact proteins when you are injecting the lcm as like your protein is always there, then for sure we do not get any you just go and clog your nano (Refer Time: 51:56) something like that.

So, you have to quickly check that my protein is fine. So, I am going to the LCM as. So, like this as a quick check point at the different experiments like NMR (Refer Time: 52:05) any kind of things you can always use this as a quick tool to know the protein quality that is the main purpose of this. And cross check also like 3 minutes like you know it hoarily matters like you know 6 samples you are giving the data as sample just you are heating to hundred cooling it up that is it the same (Refer Time: 52:26) working on DSE or CD or this kind of a techniques the whole mass spectrometry actually fine. So, that is all anything else?

Student: (Refer Time: 52:44).

We will quickly (Refer Time: 52:46) 3 minutes I will search you load one sample quickly (Refer Time: 52:49) done there Suchi.

(Refer Slide Time: 52:54)

So, you just look one sample first. Just we are showing the previous data to get just get understanding because we have only one PSA. So, like you know you can clearly see there is a change in the TMs like you know T i points like whenever they are interaction generally you are always stabilize the complex. So, there will be a shift in the TMs.

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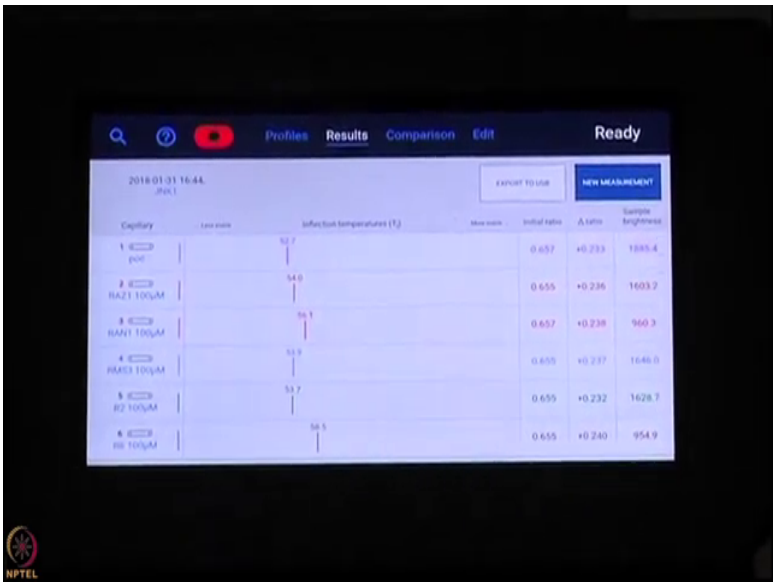

So, typically you always get a curves with a clear TMs I mean if you just look at the results you can always see like you know you always get a temperatures at which they got unfolded and its clearly showing a shift over like you know somewhere 3 degrees somewhere 4 degrees. So, like this you can always see the differences in the TM.

So, that is the biggest advantage and other thing is you are seeing the stability of your protein for always you know like many times you purify the same protein over some months or something like that. So, you can always compare like you know what is the profile similarity what is the percentage loss I am doing it. So, this kind of all things you can always keeping them in your work flow to measure the protein stability.

So, you always get you know the delta ratio which should tell you the percentage unfolding the sample brightness it is not exactly not a quantitative parameter it is kind of a relatively like you know.

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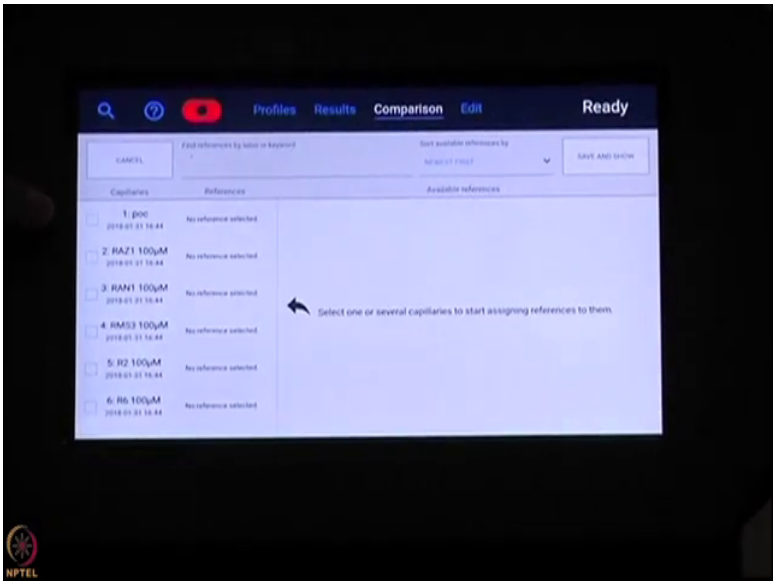

If I am taking one sample standard against that one what is the brightness what you have it.

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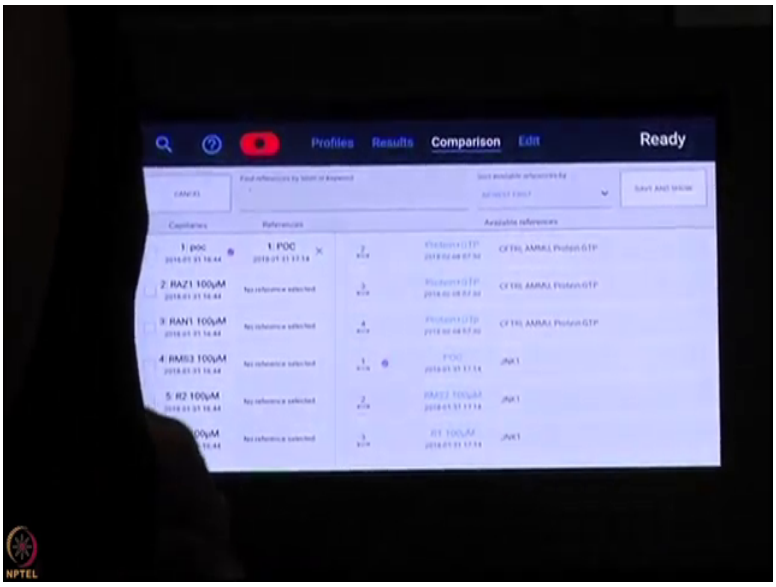

Like it always give the relative quantitation figure also. Since very easy like you know as I said like you know point where you can always assist the protein quality for before going to any bio assay it can be SPR, it can be like you know any ITC anything like that.

So, you can always quickly know the quality of your protein whether it is really good or not and if it is good then compare it with your references how it will actually works on that is the biggest advantage what you have. You can have one sample is not make sense to show you all that the data, so we are showing you the previous.

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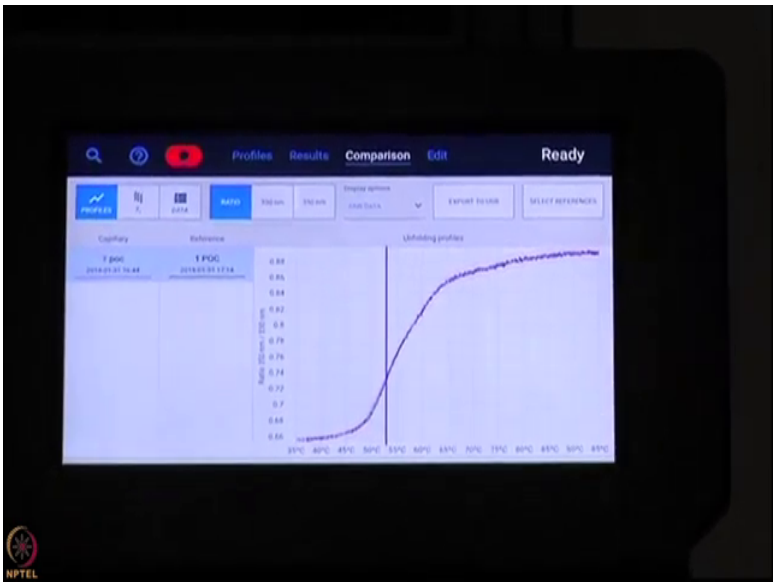

So, this is how typically you are getting it here software automatic calculator and then show you this is the TM filter like this.

Student: Because the analysis having 2 to 3 proteins in a same sample (Refer Time: 54:48).

Then its that it all depends on like you know when it is actually unfolding.

Student: No.

So, in some the tryptophans might be exposed some tryptophans are like already buried inside. So, in principle you get always very non-specific data now its a biophysical always probably the few protein is recommended, but yes many of our customers having two proteins

they have tried it. So, always they know like you know 1 to 3 TM like they know rather than whatever it was getting for the other protein like this, its always like (Refer Time: 55:17).

Student: (Refer Time: 55:17) 200 in the glass (Refer Time: 55:18).

Sorry.

Student: We can see that.

Yeah absolute, absolute and you can always see the multiple TMC in the same protein it can have that you know same protein have multiple domains which are like you know exposed at different temperatures like particularly maps if you see you get the fabrigen and FC region at different different types. So, like this even if you have same protein (Refer Time: 55:37) multiple TMS you can still get the data.

So, this is the biggest demo what we can do. So, this is how you can get the typical results and all these stuff. So, any questions something like sure like you know your proteomics means everybody will be doing some kind of purification mass spectrometry all these kind of things. So, we shall be very glad if you are interested we can evaluate and we can still give more insights to the technology.

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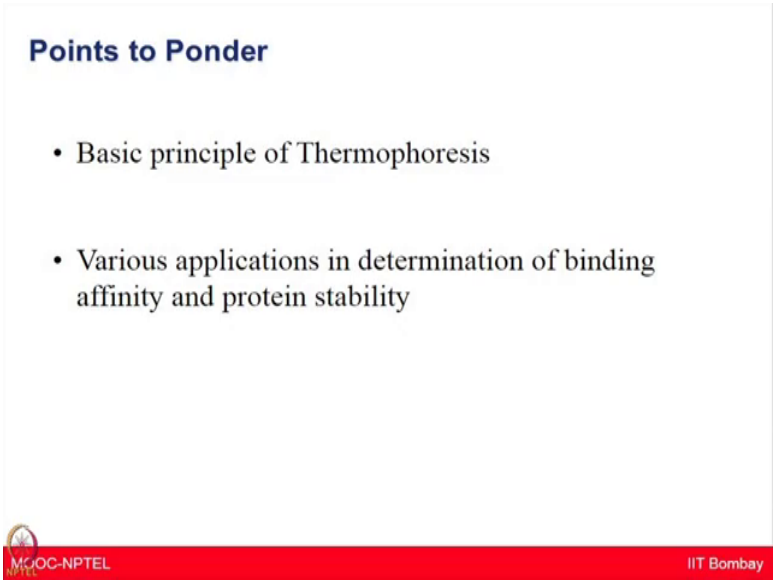

The advent of new technologies both label based and label free have now started offering us new insights for lot of new functions for the protein of interest for which there is no function defined earlier. In this light there is advent of new technologies which aims to do these experiments without tacking the protein of interest and also provide the binding and the kinetic information.

In this light there are new developments new technologies are coming forward and today we try to provide you interaction with one of the leading companies and their application scientist who talk to you about MST technologies and also the quality control assays we are done using Tyco technology. I hope these provide you some insight about how to do your experiment for the protein to further characterised using latest label free biosensors.

Thank you.