**Interactomics: Basics and Applications**

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**Lecture - 43**
**Applications of Label-free Technologies-II**

In the previous lecture you obtained a glimpse of the principle of different label free technologies and their possible applications. In today's lecture we are going to talk about the recent advancements in the field of label free techniques and how one could integrate different technology platforms. Imagine that we have talked about mass spectrometers, we have talked about SPR, we have talked about BLI.

Can we start comparing these technologies, can we start integrating these technology platforms this is what we are going to talk in today lecture where I am going to talk to you about SPR imaging, one of the latest high throughput platform which can do a similar thing like SPR, but much more in high throughput manner, integration of SPR with mass spectrometers for identification of protein interactions and some of the pros and cons and comparison of 2 major technology platforms for label free biosensors like SPR and Bio layer interferometry.

So, my today's lecture I hope is going to further unfold the applications of label free technologies in analyzing different molecular interactions. So, let us continue with my lecture which I had delivered in this workshop.

So, SPRi, SPR imaging is one of the high spatial resolution platform which allows for high throughput analysis of various type of biomolecular binding interaction which we want to measure.

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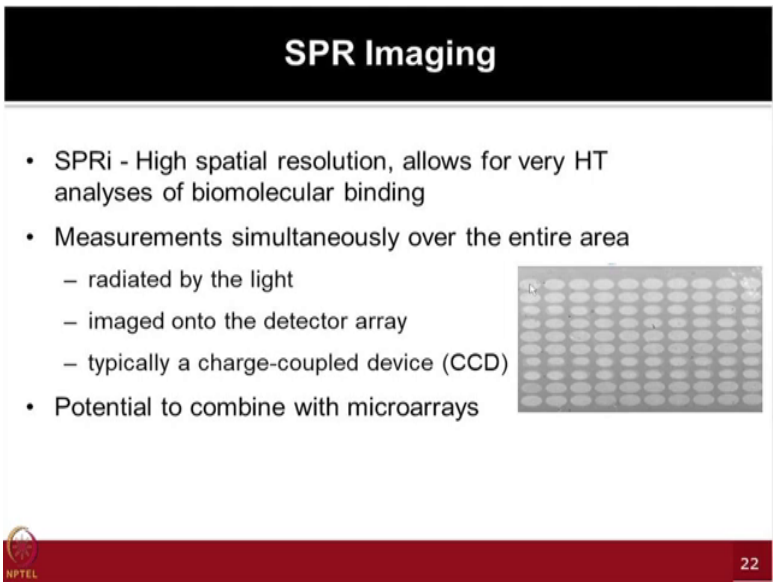


Here you can intentionally that you are using imaging platform. So, imagine that you had the gold chip which was we are using for a SPR, but in SPR gold chips we have the very small region where the gold part is there. Rather if you have the full chip which is having the gold and then now you are using imaging to image the entire surface.

And then that image is going to generate some sort of pseudo image like a microarray which will show you that you know each one of those feature which you have printed on the chip will give you an image which looks like microarray, but it is not actually microarray. So, that kind of concept was used, can we image the whole chip rather than you know exciting into on a specific angle only which what you use in the SPR case.

So, you are radiating by the light imaging on the detector arrays, you are using some sort of CCD devices which are not so costly. And then this particular platform showed potential to

combine with the microarray based technology because anything now which you can do in high throughput way for the whole chip is very much compatible with the microarrays. So, this is where you know one of the image, I am showing with one of the experiment I was doing in Josh lab earlier when we were developing some SPR imaging based platform on the early prototypes.

So, as I mentioned to you; you are seeing something here just the pseudo image, it is not the actual you know you are not (Refer Time: 03:18) fluorescence. These are some of the pseudo image obtained from this kind of platforms which shows you compatibility with SPR imaging.

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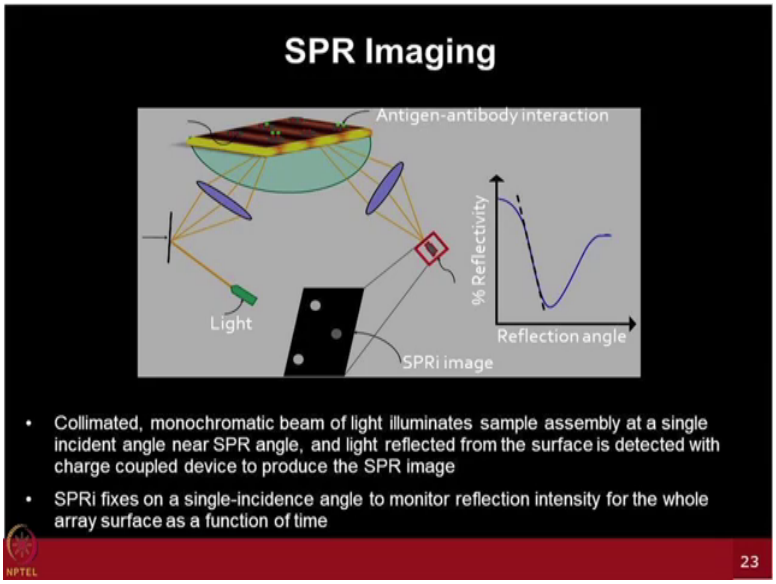


So, what happens here conceptually? We have this prism, we have this gold chip, but now here we are exploring the full chip potential to scan the whole surface here. And then by

generating this SPR image again the concept is same, we are still looking for reflection angle, we are measuring the percentage reflectivity change, when you have too many spots printed on the whole chip surface you need to find out some common angle which can be best angle for doing this SPR experiment.

And that is where you have to play with the conditions to find out what can be that best angle which can be fit for the whole chip surface to do those kind of process. Once you find that and again that may not be the best and most accurate angle, let us say 19 you have decided or 20 you have decided you may have to play with more to find out what can be the best angle to freeze for the whole chip to be scan.

Nevertheless the idea here is to generate the pseudo image of whatever is printed on the chip without adding any kind of fluorescence labels, you are just generating the image on the gold chip.

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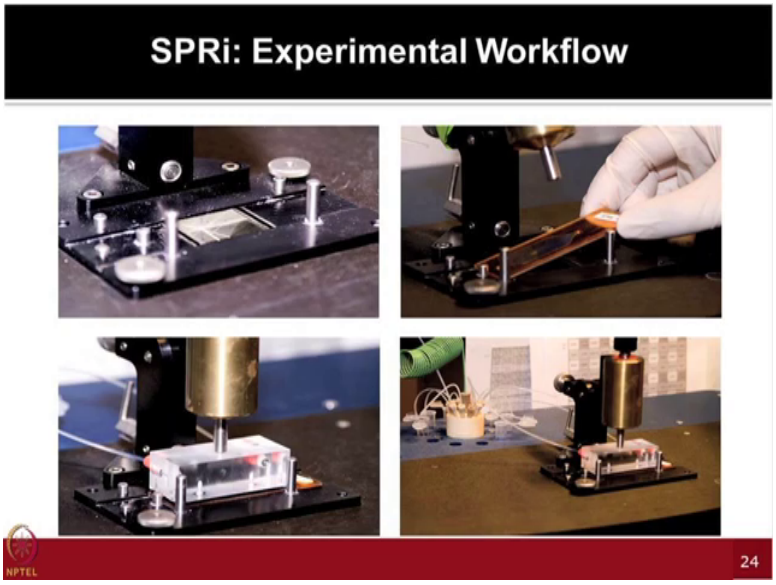


I am showing you now one of the workflow which was done several years ago, which was on a prototype to give you feel of you know how these can experiments can be done. So, this is you know prism here and this is a chip full of the microarray kind of chip; where you can add all the you know protein feature which you want to analyze. And there are certain holes on this you know on both the sides and these holes you can now combine with the flow cell from which your liquid can come and pass which you want to test out.

And then now you can screen the whole chip surface on the SPR kind of setup and it is you know just reminds probably Josh that you know one of the old setups we have the prototypes, where now you can test 8 antibodies for example, or 8 different proteins in very much to you format like you know you are developing a new instrument setup and this kind of you know

solutions are coming in. You have the gold chip everything is you know passing from those and then you know it is going back in the washing containers.

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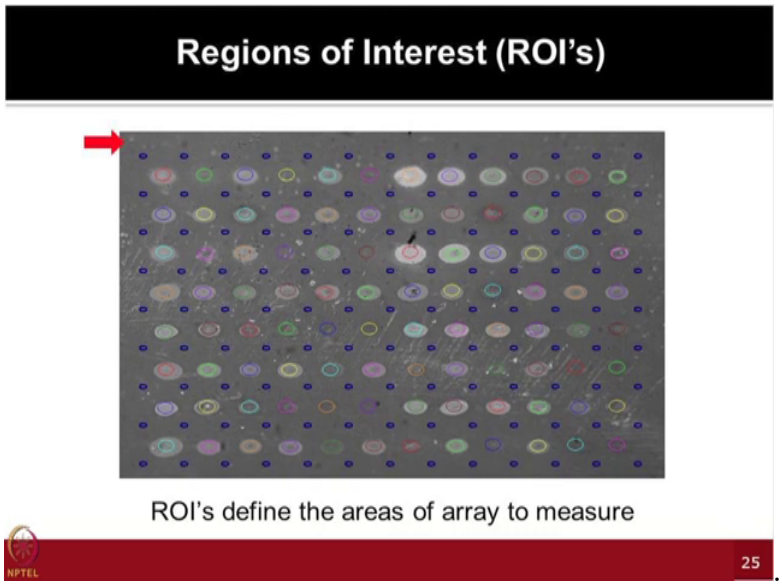


So, these kind of platforms could be used and it result into these images which are the pseudo images as I mentioned to you, it shows you these kind of a spots. Now all these red green what is shown here these are actual spots, but along with them whatever you see the gray color is the widget background. So, software are artificially add some blue color dots these spots could be used for subtracting the background.

So, in this way now you are ready to test lot of molecules unlike the for what you can do just by using the (Refer Time: 05:45) technologies and these are known as the regions of interest ROI's. In any kind of micro array experiment, any kind of a SPR, imaging experiment you have to ensure that you know what you are measuring is the right signal and not just kind of

you know that particular feature what intensity it has. So, you have to take into consideration the intensity of these spots in combination with the background what is there.

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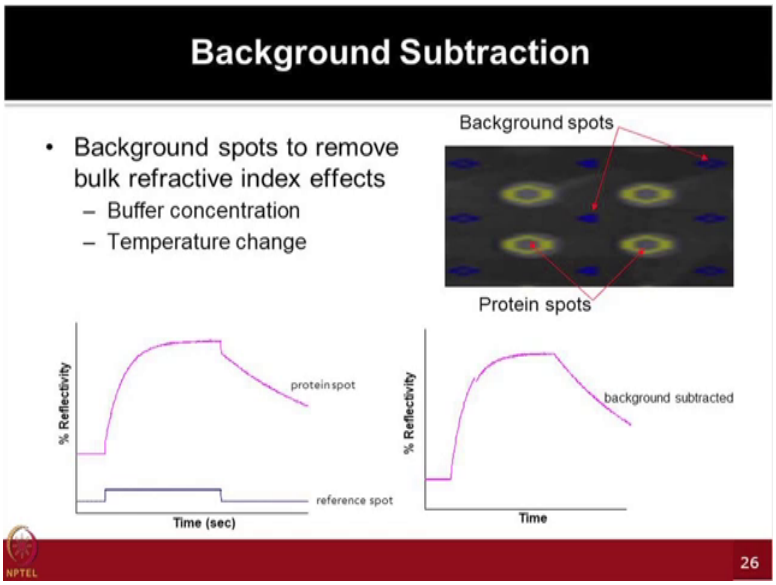


So, therefore, for each of these spots we had four of the reference spots; so if you subtract the average of these four from this particular one and same you do for every spot then probably you are only measuring what is printed on each of the features right. Then you have removed all kind of side effects coming from those chips and then now you can see that how much difference it could make.

For example, this was the actual spot which is showing you the binding and now this is something coming from your blue spot because you know buffer is flowing on the whole chip. So, there is some sort of you know bulk effect you will see.

Now if you subtract this then your shape got change. Your sensorgram looks little different now right. So, these are the kind of thing which can make the your curves very different, if you are just not using normalization or if you are using those background subtraction which is very crucial.

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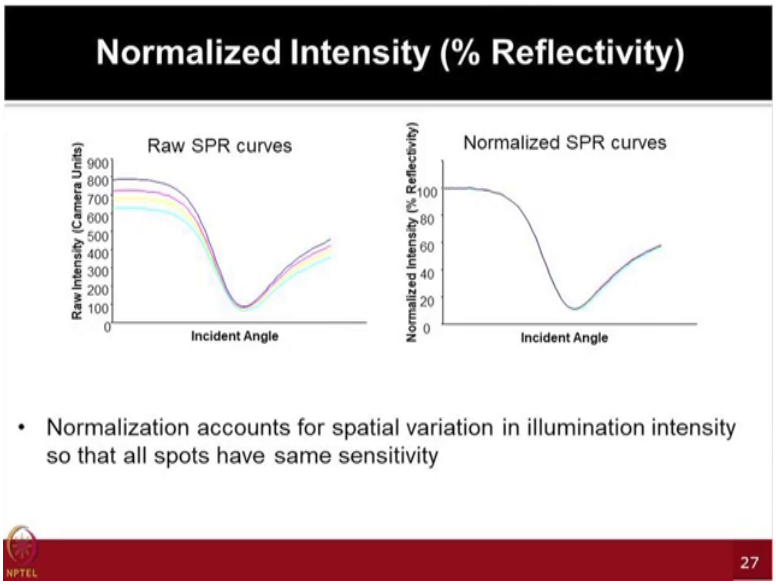


Why should really normalize these features on the chip surface and becomes more critical here because you have many spots printed on the long chip and imagine that you know when the liquid is flowing from one side and reaching to the other side, it will take some time. And you are you know you are start seeing binding for some molecule and then eventually you will see for every other molecule on the chip right. So, then they will show the different type of time at which the binding started, but ideally you want to compare all of them together.

So, then you have to normalize again them, so that now everything whatever you see, so, it was coming from you can see the intensities are different looking here for different angles. Now you have normalized the SPR curves and everything is now looking same then now you have opportunity to compare their signals. So, the SPR signals which is we talked about sensorgram they are the one actually if your eyes are pretty expert in tune to look at the data immediately start telling you lot of information.

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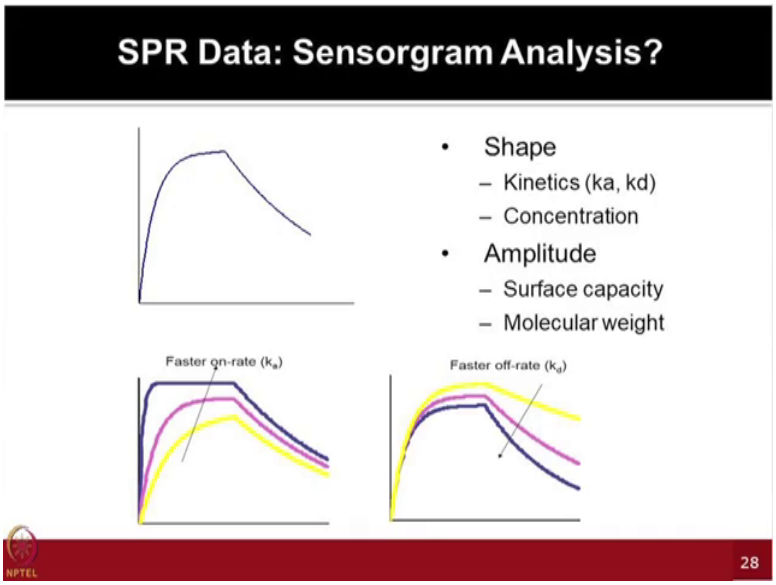


The SPR data just you know it shows you the different curves will tell you that you know what is happening for the kinetics how much concentration I can see for those proteins, what is the surface capacity molecular weight etcetera. And many times you know these kind of curves which are showing you the just the difference in the on rate and off rate can be very powerful. Because imagine that you know you are working toward developing different drugs, and objective for you know those drug development probably I would have quoted the

several examples. If you want to really make that drug work very fast right, you want immediate effects for you know any accidental condition any kind of you know headache kind of thing you want just very quick response.

So, your on rate you are looking at that time versus your off rate becomes very different right. So, you want immediate action to happen something has to go by in very quickly.

And then if you are looking at you know the sleep response you are looking at long term response for a given particular drug, then you are thinking that let it go off slowly, you know you do not want it to just you know show very quick binding and release you are thinking about it should go very long. So, that you have more effect for that particular molecule. So, both of these conditions probably can give you the same kd value, it will show you same kind of response overall, but on rate and off rate could be different and those can be so, detrimental or useful for your kind of discovery you know portfolio which you want to develop.

So, therefore, these kind of you know curves are very crucial as you are watching and there are many companies who are work with us who do the testing in our labs in our facility, who are trying to look at many of their you know the biosimilar products and some of these kind of testing they do. We can see from their experiments that you know just looking at the shapes of these curves of sensorgram can make them feel excited or not so excited. Because immediately it tells them it is working or not working.

So, all these kind of stuff is just visually you are seeing of course, you need to do better fitting of these curves to find out the actual values for concentration or looking at the kd values, but you know many times these experiments gives you lot of visual feel of doing those analysis.

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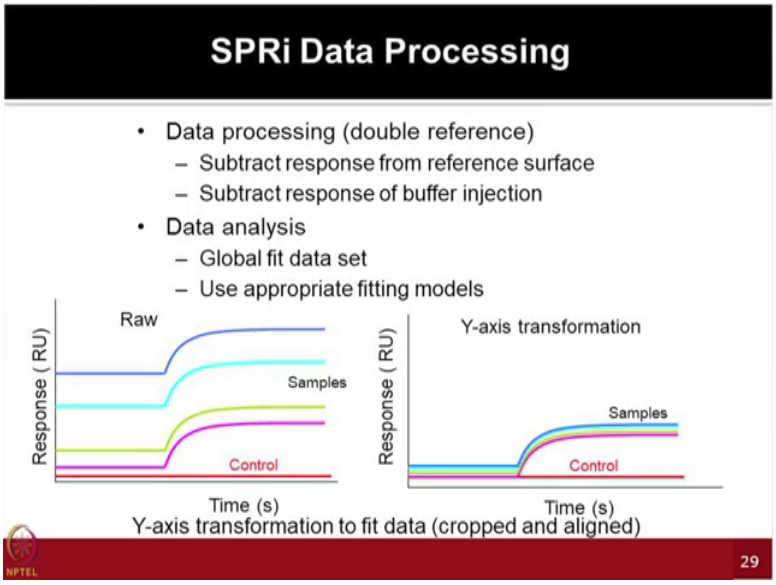


But as I said you know if you want to really get the proper values you have to do good cropping, you have to do good fitting. In this case for example, as I mentioned your response timing is different because you have different molecules printed on the whole gold surface where you have the some starting right now and some starting later part. So, then your response units are shown differently here, but now you are normalizing all of them and then you want to see comparison of their responses throughout the whole experiment.

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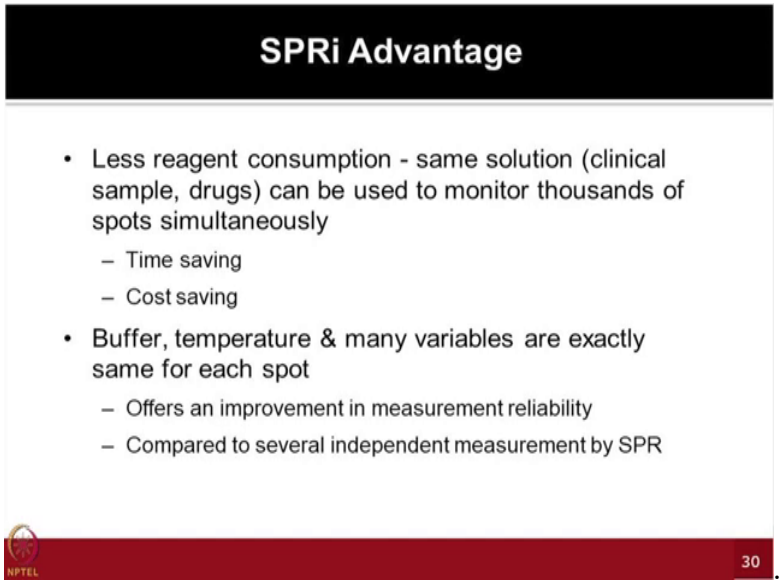


So, therefore, data processing becomes very crucial, but advantage of using SPR imaging a like high density microarrays where you are using less of the analytes which you want to test. In the same kind of sample which you have available especially the drug molecules which are very low in quantity or some other clinical samples, I think you have opportunity over there to now test out on hundreds of features simultaneously right with SPR imaging platforms.

So, that is something your time saving cost saving of course, and going to be much more reproducible experiment. And then within the same type of condition which you provide, now you can study the behavior of lot of molecules under the same condition which is very critical.

If you are varying the you know chip to chip there with some variation and the day to day temperature variation lot of things, even you are doing under controlled condition, but if you split 100 analyte testing on 25 chips what is doing on one will have huge difference.

So, buffer, temperature many variables are there and if they are on the same spot at least you are you can neglect them you can normalize them, you have a better ways of having the controls to negate those kind of you know negative effects from coming from those molecules alright.

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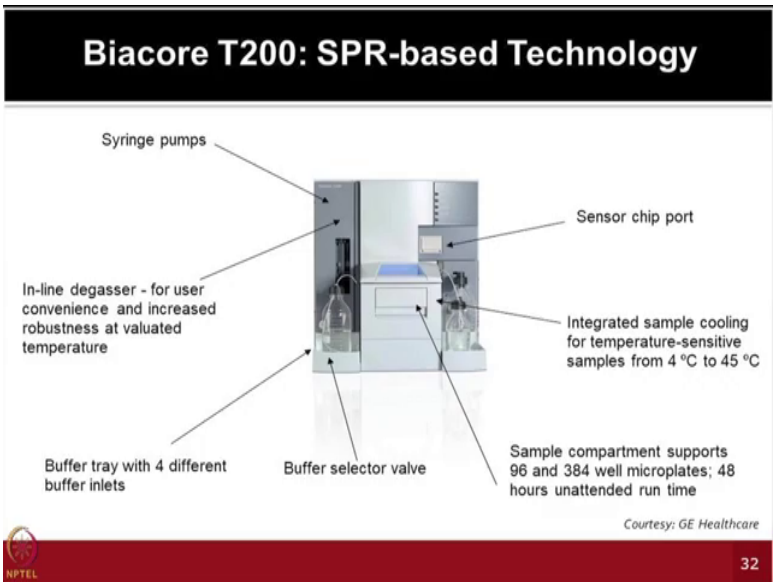

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So, latest popular technologies: one of them is Biacore T200 is one among them offered by G healthcare. There are many type of chip which are coming from these particular type of technologies where you know how you want to mobilize your molecules, sometime you have different type of carboxymethyl based surfaces sometimes nickel NTA kind of chip can be very useful for you if you have his-tag based proteins.

So, you can select the right kind of chips for your use and accordingly you can make use of them. Biacore T200 is right now one of the industry leading technology which is pretty much GLP approved. So, whatever data you obtain here can be very much sent for FDA or other kind of approvals and that is why lot of industries are looking for these kind of technology platforms.

Nevertheless you know these kind of technologies are giving you a very precise data, but they are very costly you know considering the how much cost it takes to do the experiments. So, many times if you are in the discovery development mode and you have hundreds of you know compounds or libraries to screen, this may not be the best platform to start with, because you are limited with the you know how much cost you have to screen that many large number of compounds.

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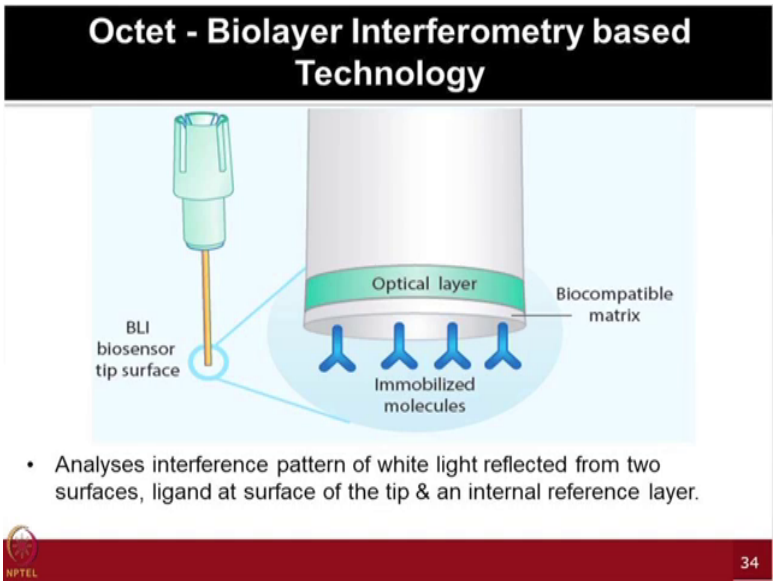


So, new technology platforms like one which I am showing you here which is Biolayer Interferometry based platform can be very powerful for first round of screening. It may not have that sensitive on rate off rate and those kind of KD values which you want to obtain, but it will definitely tell you binding happening yes no in a very quick format using your solutions

what you have for different type of molecules to test out and here they are looking at the interference pattern.

So, on this sensor there are some you know these antibodies which you want to test out; they are immobilized and then they go inside the like a (Refer Time: 12:45) plate and then those sensors are going to go in and test out your binding using the interference change which is going to happen. So, this is something is we have a follow up lecture so I am not talking much detail, but I wanted to give you the feel that you know in which way you can move forward from different type of platforms.

So, a good thought can be you know from the microarray experiments you have got leads let us say you know 200 molecule which is of your interest now, you can go to BLI to narrow it down further. Now, you can use probably Biacore to test out you know how many of those you want to now measure the exact on rate off rate right ok.

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So, there are many label free platforms which are possible. I am restricting right now the applications of those mainly based on the SPR right now. Because that is what is something which we have currently available at IIT Bombay, and I am also running a core facility which is offering services for many type of applications for internal users and external users.

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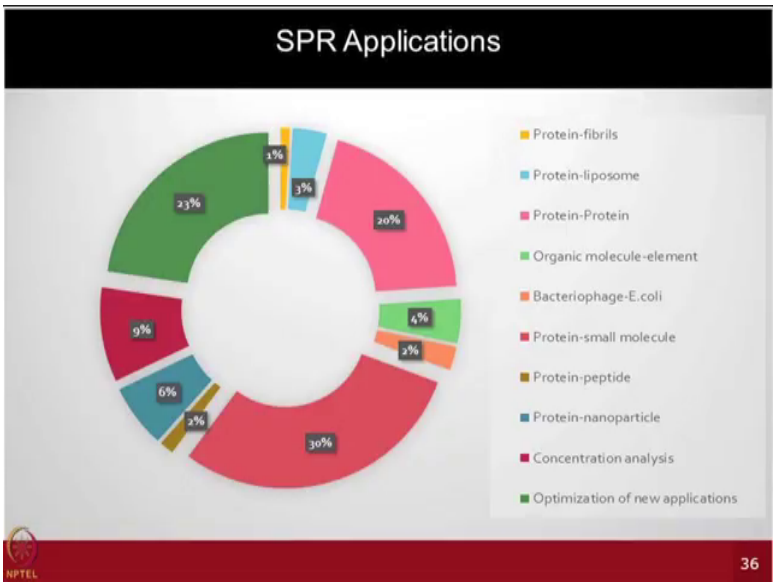


And the kind of you know this particular distribution, but what it shows to you that we are doing all kind of applications here. Looking at even from protein fibril interactions, protein liposomes, protein-protein, various organic molecules and one you know interesting experiment came when somebody wanted to print bacteriophage virus on the chip and test with the E-coli whether those a binding can be seen or not.

So, you know all kind of thoughts you can have what you want to measure on the SPR and of course, popular ones includes protein peptides, nanoparticles, concentration analysis something which we developed a new assay and optimization of many new applications. So, that is the ongoing thing, we always want to see that what more we can obtain from these instrument platforms.

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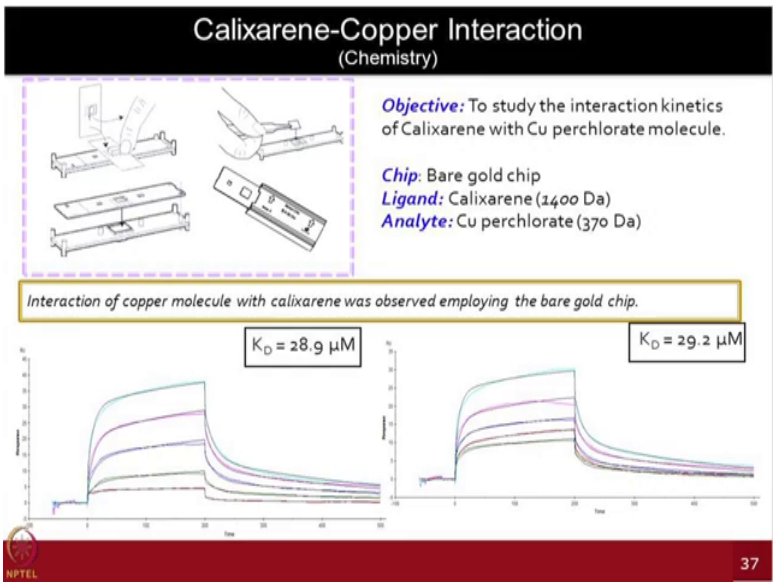


So, I am now kind of you know flashing it through couple of application slides with just an intention that what all things can be done on these platforms. These are not my results, just result coming from our facility which our people are running the experiments, but what it shows to you that you know variety of things can be done. For example, one of the chemistry faculty lab was looking at one of the (Refer Time: 14:34) molecule and copper binding. And to do that there was no chip which was available for us to do immobilization.

So, we gave them the bare gold chip, the small part of the chip and then they did the immobilization them self of those and after doing those particularly this is a step which are shown to you. So, sometime you may not have readymade chips available for the kind of functionalization which you want to follow. And that side, you can use the bare chip where

you can try out your own immobilization chemistry which could be then used to further do the SPR experiment on biacore chip. So, that is what we have some bare gold chip as well.

So, this one showing you 2 different experiment, the K D values obtained are pretty close and that was at least you know interesting experiment for us to try out because it was not the readymade chip available for it and still immobilization was pretty reproducible.

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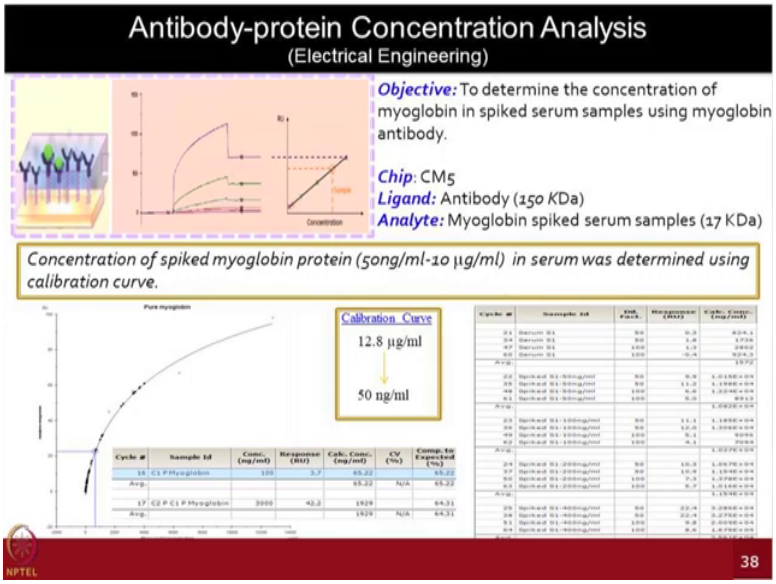


Another lab from electrical engineering their intention is to have some benchmarks. So, let us say you know Biacore technology the benchmark available in the field, if you can measure you know an interaction and generate some KD from Biacore and now if you develop another bio sensor and you can show that you know I can generate same kind of KD then probably you are talking you know really robust platform development right.

So, even for any new technology development you need to have some sort of you know benchmark development. So, therefore, what they wanted to do? They wanted to just look at the myoglobin protein binding with the anti myoglobin protein and in fact, they are looking at also in the serum now. So, when we try to do this thing we were able to pretty much get good concentration of this myoglobin protein and that something which made them very happy; because now they can test their own biosensors and see the benchmarking how close these values are.

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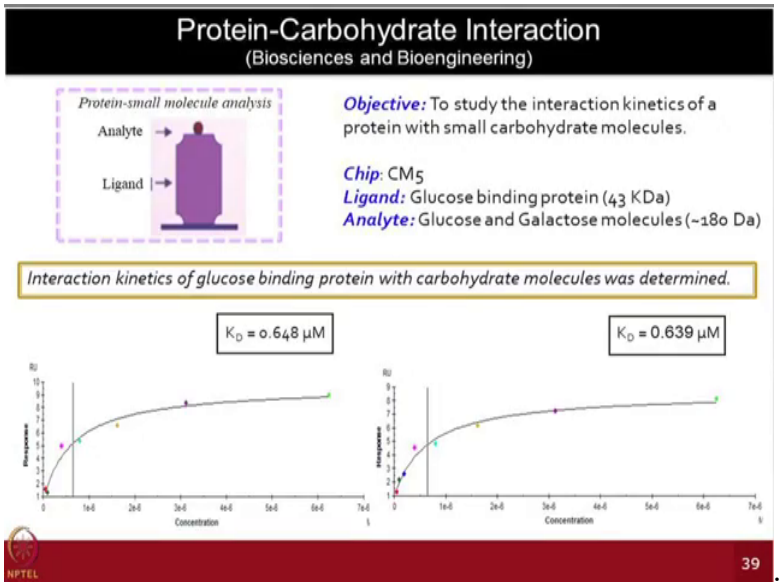


Another experiment test from the biolabs here in the protein carbohydrate interaction which is one of the very common thing which we see. Many people want to look at it local binding protein, and different type of carbohydrates binding to the proteins. And again you know why I am showing you duplicates because it is become very crucial to give us the confidence; the different experiments are giving the same K D values. Again I am not talking the biology of it

probably I should not talk is others word, but I am just giving you the feel of variety of applications which are possible by doing SPR kind of technologies.

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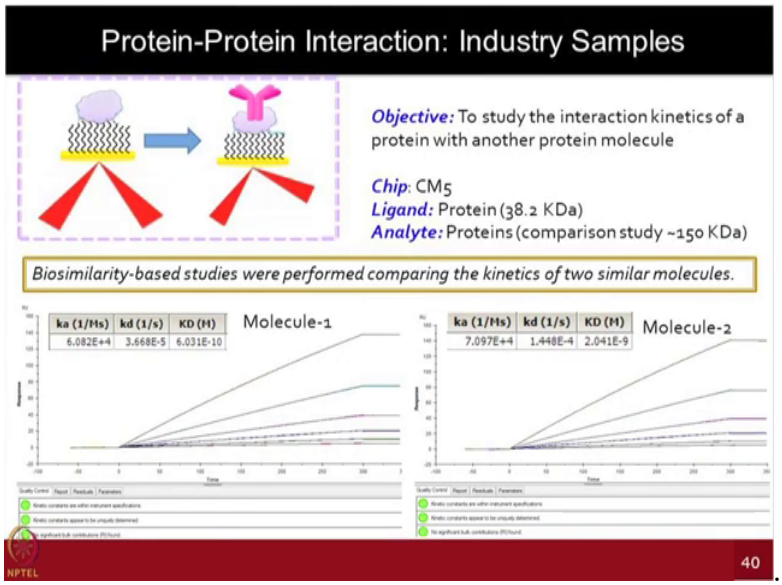


Now, one of the industry sample which of course, I cannot disclose which was trying to compare that what data we obtain on Biacore, how would that you know similar or dissimilar when they are running to their parent company somewhere in Switzerland. And they had that kind of numbers and the you know KDs in their mind and they wanted to compare the things here and those molecules showed pretty close binding based on these Biacore experiments.

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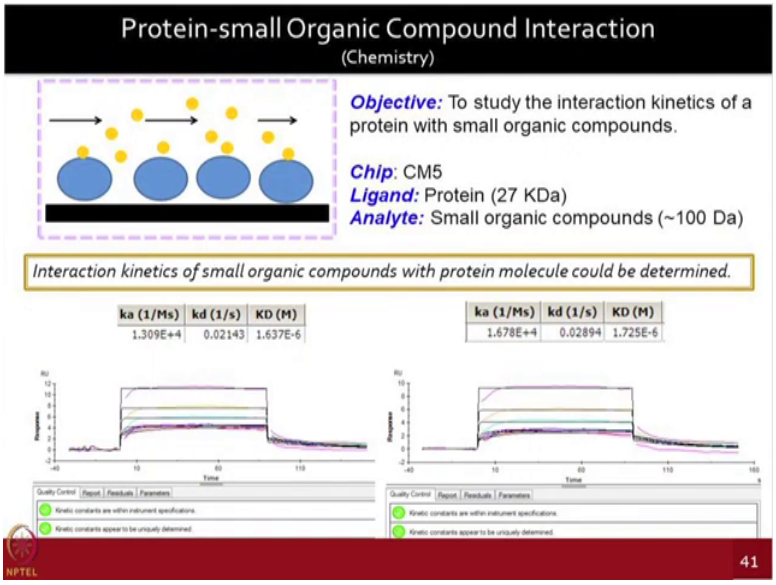


We also have some faculty from chemistry, who are looking at protein small organic compound, their interactions. In this case one of the unknown protein which is not known to us 27 KD protein was immobilized and then we are looking at how small organic compounds can bind to them. So, again many of these times the KDs are very close and that is giving us you know good confidence, that these experiments are you know in duplicate are working well.

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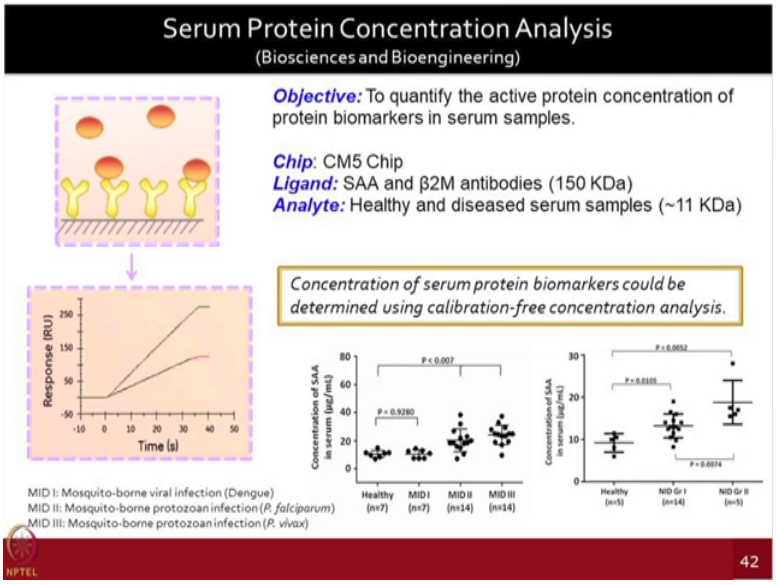


One interest which we had in our lab was to measure some of the known protein concentration in the serum. For example, from our discovery approach of using dyes and (Refer Time: 17:30) based platforms couple of protein we identified which looks good to us and as the potential biomarkers. So, we thought can we measure the level of those proteins in the serum using SPR and to do that you know; some new assays were developed which was you know which we termed as the concentration free calibration analysis which was the CFCA analysis where you know different type of in you know patient from this is one of the malaria project when we had patients suffering from either falciparum or vivax malaria or dengue fever.

And we wanted to measure the level of one of the proteins serum (Refer Time: 18:02) and we wanted to see that in how that protein concentration is different in different type of conditions. And because we already had idea for this protein from our other data set discovery

data set. So, we felt pretty confident that you know we can now measure and this is what you need you know you cannot do validation on large number of samples using mass spec or other type of technologies. So, then you have to come down to either ELISA or this kind of label free approaches which could do screening of you know the same molecules in a very high throughput manner.

So, this was a good experiment for us to show that you know we can actually measure the concentration of this protein of interest to us. Again you sometime you have to develop these assays in the beginning. So, you need your protein its good idea to try out something if you are planning to do these kinds of experiments with the known proteins for which you have the purified proteins available. Build the confidence and the assay conditions from those then you can apply for some unknown compounds as well all right.

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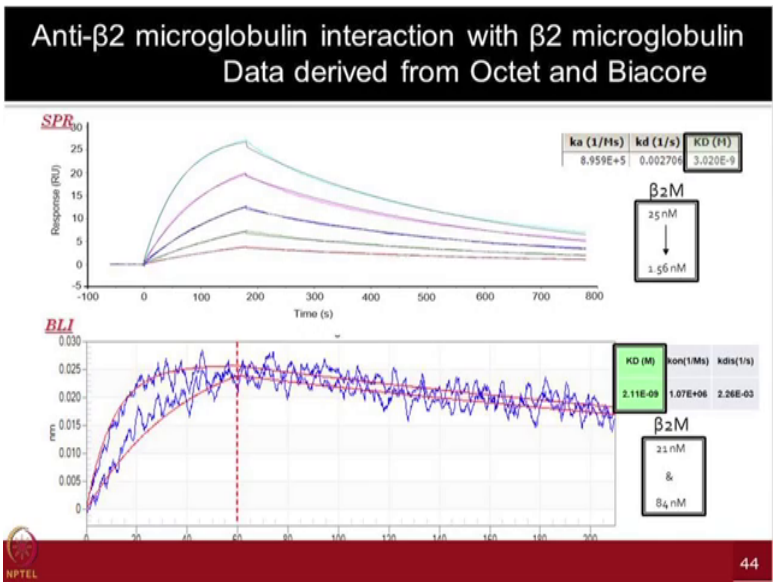


So, I am not showing some data actual data from 2 different type of technology platforms. So, we compare for couple of proteins both BLI platform and biacore T200 platform for few experiments because you know the cost wise they are very different, but sometime the you know looking at the acceptability wise things are different when the kind of GLP compliance while the (Refer Time: 19:13) platform is more compatible.

So, can we use this you know at least the BLI platform as the first level of screening with high confidence? So, in this case we use anti beta microglobulin and this antibody and the protein interaction. So, using SPR and BLI we tried binding for various time points analysis which we did different concentration series, came pretty close actually you know this is showing you 20 the overall KD it is almost 3 e to the power of minus 9. And it is also 2.11 e

to the minus 09; this since just imagine was done just you know in a like this kind of estimate what is being brought here not available in my lab that time.

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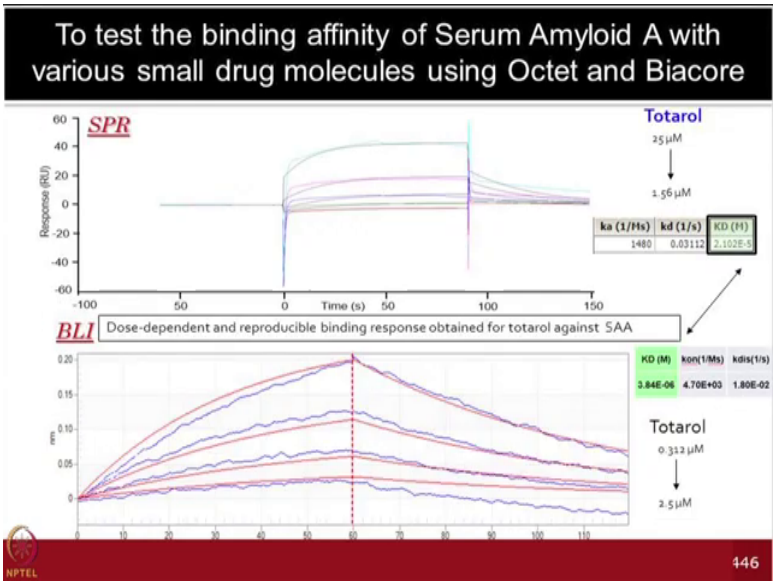


So, not in the best optimized conditions, but still the way I can see there is not huge variation; just the measuring these kind of you know the abundant molecule bindings. Then we looked at some of the you know compounds of interest for a given project when we had some drug totarol to look for their binding and again things are pretty close you know coming from the two platforms available.

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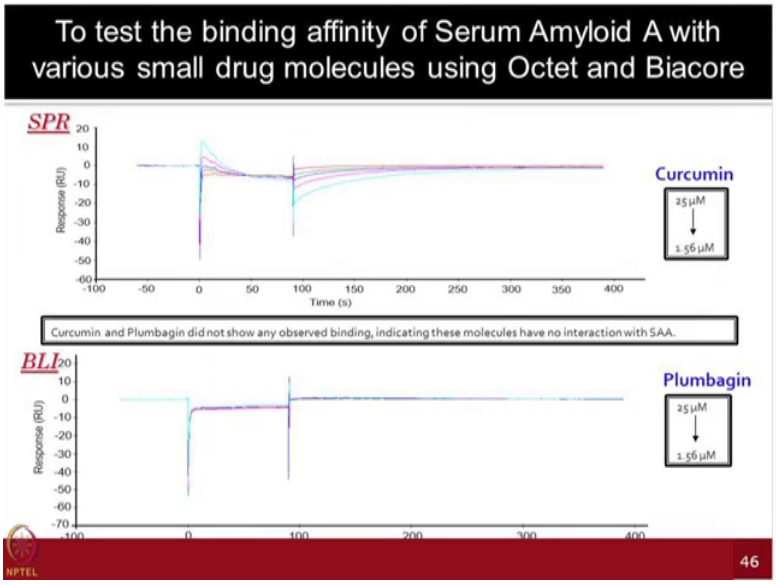


We also tested you know another drug which is shown here Curcumin and Plumbagin which we are testing for some bacterial protein against those and we are seeing you know the changes that are pretty close coming from both the platforms. So, you know what I want to convey you that you know many times just do not just rush for one technology platform. Think about if you have to test out many compounds what can be the cost for doing that what can be the variations of doing those experiments. Start you know using those platforms which can narrow down your leads and hits, then do the final experiments on something which is really you know acceptable.

And of course, if you have huge amount of you know funds available for doing that project then you can do everything on the same platform, but otherwise you can mix and match and choose which technologies can give you what. Sometimes you know a good experiment may

involve every technology which we are you know studying in different workshop right now here.

Starting from you know discovery from mass spec based stuff and microarray based stuff you know take those leads forward and then validate using these kind of label free platforms. So, everything you know has its own utility and you have to pick the technologies in the right context alright.

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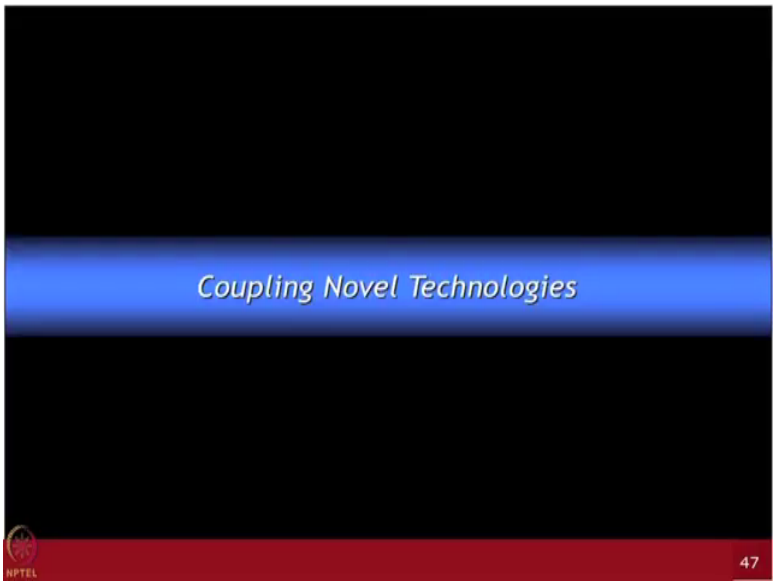


So, now over the period what you would have realized some various lectures given by Josh there is so much need for having integration of new technologies and development of new technologies. And there is a coupling different existing platform becomes very crucial.

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In Josh lab we thought couple of years ago and many people are still working on developing those technologies of coupling NAPPA technology with SPR imaging based platforms.

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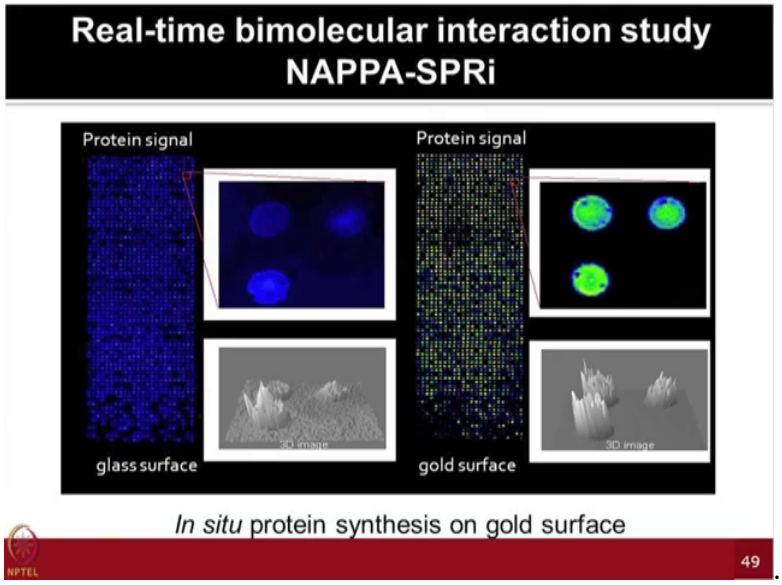


The initial experiments done by Manuel Fuentes and then I joined the lab where actually you know these are you know quite old NAPPA chips 2006 2008 that kind of timing. So, the glass slides are showing these kind of signals which I am sure by now you have seen this, it has improved tremendously. And then the same features printed on the gold slide are showing much better much more intense signals. So, first of all the objective was to see whether NAPPA concept can work on gold or not.

Because so far to all done on the glass slides and first time it was you know planned whether we can mimic the same thing on the gold surface, but looking at the 3 D view then AFM images it was I think you know apparent that probably NAPPA could work on the gold surface as well. But the traditional NAPPA chemistry which you are aware of having you know many components in the master mix right, which included you know BSA, BS3

cross-linker you had the capture antibody and of course, your clone of interest the cDNA of that having GST tag.

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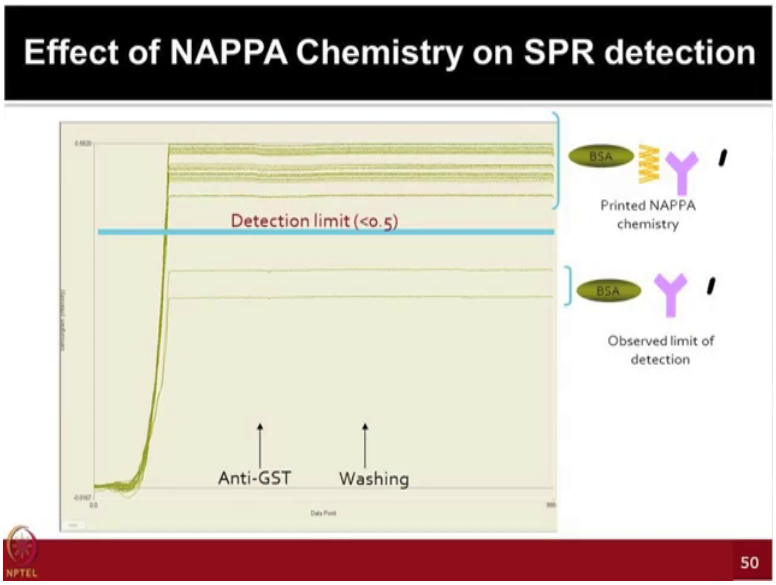


So, all of them were actually making the whole mass too much which you are adding on a given chip surface right. So, the initial experiment when it was started the baseline when you are printing so much mass on the gold chip and in the SPR kind of platforms you are only measuring that how much binding you can see happening right. So, to begin with itself for the baseline itself is going of the response unit.

So, here you have literally no room to find out how much binding you can observe later on. Because with the buffer alone the base line itself is very high which is passing beyond the response unit of the instrument which is SPR imaging platform.

So, I talked to you about you know briefly about a technology which is SPR imaging you have been exposed for NAPPA arrays think about the combining the 2 to hardness the both the power. So, if you add the anti GST antibody and you want to measure the binding of anti GST with the GST protein then probably you have no room here to see the binding where the binding is happening because it is already passing beyond the response unit of the equipment. So, the next thing was whether the same NAPPA chemistry can work here; probably not.

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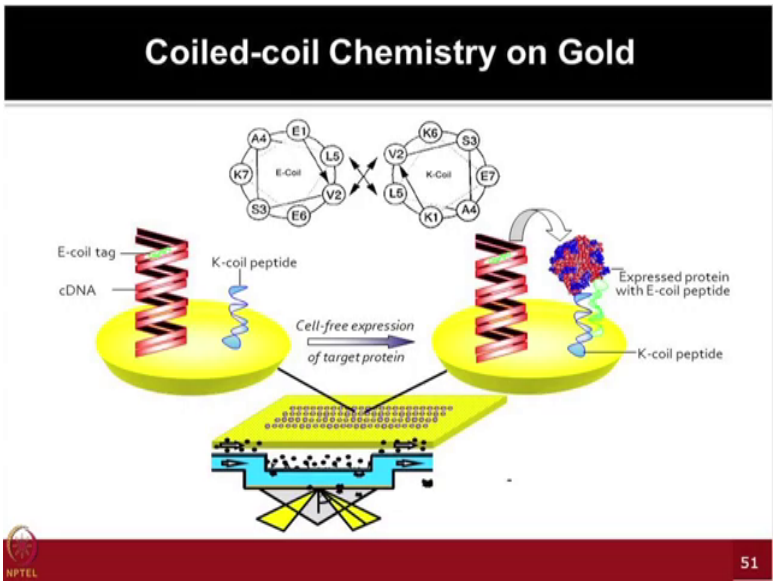


So, then there were you know a newer approaches came which was one of them was coiled coil chemistry of using E coil and K coil can our clones may have you know E coil and the chip may have the K coil and using those particular type of chemistry. Now if you chip you know print your all the clones using the same NAPPA concept, but now you have the K coil peptide and the capture peptide and each of the clone contains E coil tag.

So, you are not talking about peptide-peptide interactions here and it is a very you know strong interaction not having so much material printed on the chip surface. So, you have you know the same issue what you have seen earlier probably could be resolved by using the new chemistry of NAPPA. So, now, if you had the cell free expression system on the chip itself and imagine everything now you are doing on the gold slide. Earlier what are you doing on the glass slide, but NAPPA chemistry is modified with the E coil and K coil peptides.

So, now, if the proteins are being formed with the you know this interaction of E coil and K coil peptides, the heptamer is being formed, now you will have the tight interaction you can see this protein synthesize on the chip surface and which could be then detected and used for the further interaction studies.

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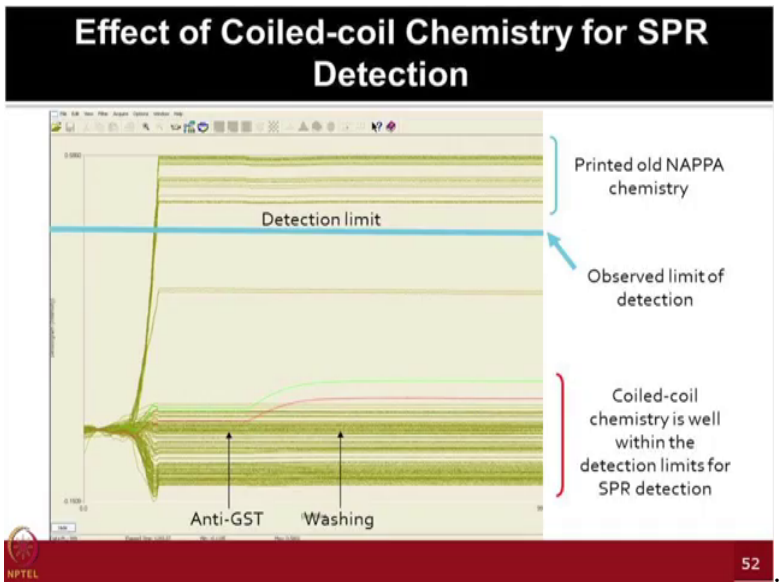

So, now the baseline which was seen here earlier, now the same baseline is much lower here with the new chemistry right. So, the old chemistry, versus the new chemistry and now you can have much more room for measuring any kind of binding responses you can change different concentration and you can still see how much binding you can observe. So, this case were only anti GST which was injected for testing how much GST proteins are being synthesized.

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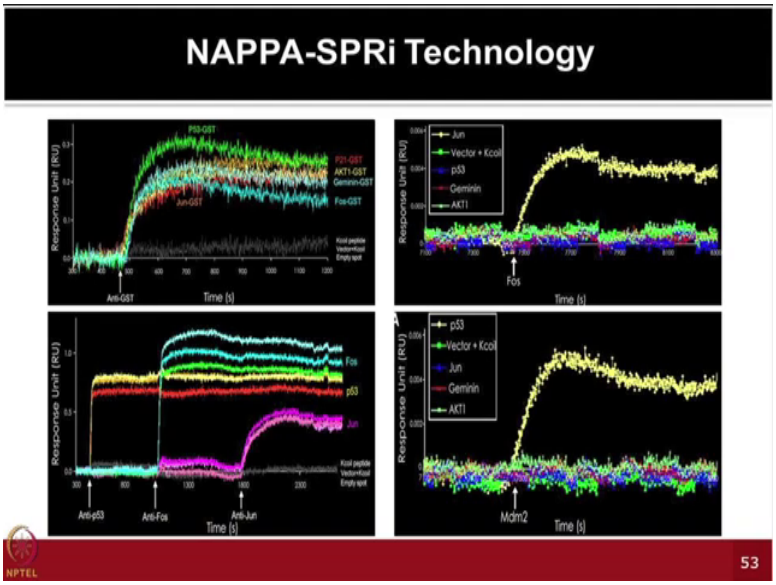


So, several experiments were done. In this case initially you know various type of proteins were tested for expression first of all; whether on the gold chip with the new chemistry can we detect the protein expression. So, every protein has the GST tag; so in this case you know handful of 8 or so protein were tested out and you can see there all of them are showing binding but is that the real binding or the bulk effect you have to negate those possibilities.

So, another experiment was done where sequential antibodies were injected; starting from anti p53, anti Fos, anti Jun antibody on the same chip. And now if you can see that triplicate spots are showing binding in response to those particular antibody which you are injecting then probably this binding is not bulk effect, you are what you are measuring is protein specific antibodies you are using.

So, protein specific responses you are observing right. In case of GST it can be something you know everything is showing you response. So, probably may not be very confident thing that it is proteins are always being expressed, but if you are using a specific antibody for p53, Fos and Jun then probably you have a specific type of you know measurements which you can make here.

And then you know some protein interactions were tested using Jun and Fos as a pair, Fos was injected and Jun was you know printed at the cDNA on the chip and there was some binding observed. Similarly Mdm 2 and p53 were tested out and then you know those kind of things showed some binding.

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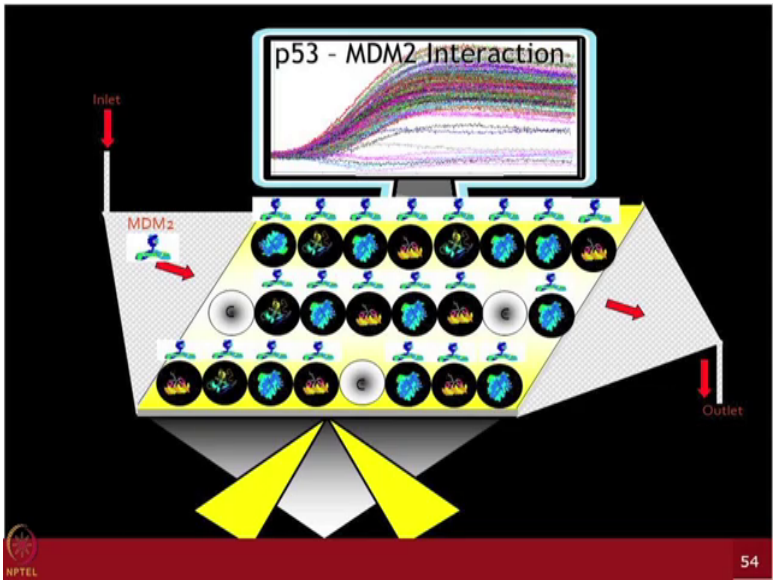


So, you know a next experiment was just planned which was more kind of high throughput experiment and which was very challenging to really do that thing on the prototypes is what we had available. Where we have you know from one of the companies in Cambridge that time gave lot of clones of p53 mutants and these are all hotspot mutants of you know different p53 regions. To test out into how MDM 2 protein it binds to the wild type of p53, what is a different type of mutant forms of it and our this is just a cartoon image just to show you we are trying to look at 144 spots binding how that can be measured simultaneously.

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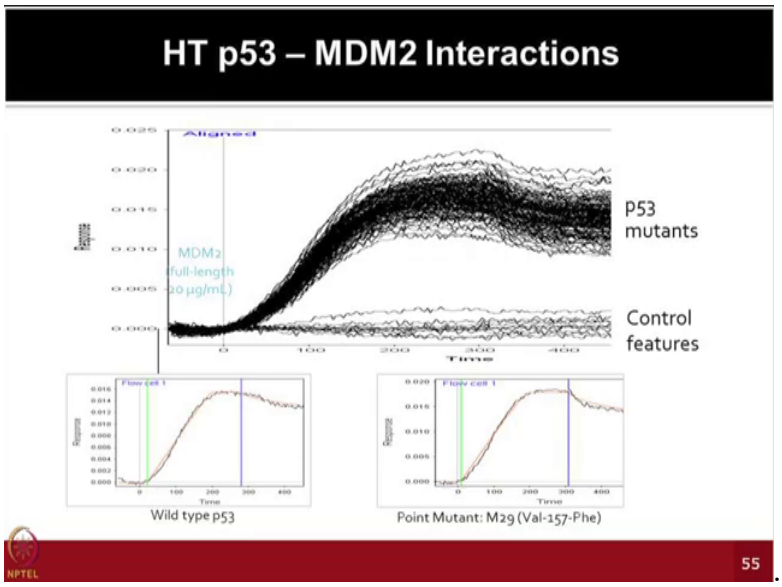


Once you do the experiment it was very you know first time high throughput type of experiment being performed, 144 spots binding you are trying to observe simultaneously. It was very complicated because there was no software available that time which could do normalization, which could process the data and there was you know lot of issues especially you were also finding that you know probably there was some sort of bulk effect we could observe. Even in the control spot there is some sort of you know binding which one could be observed.

So, for the next steps of these kind of technology development which is even that time we were seeing that even for NAPPA chemistry there are you know the halotag chemistry which you are seeing now is showing much robust signal. So, could we now try out the same thing on the you know an SPRi platform using the revised NAPPA chemistry and that is where now people in the Josh lab are following up the work which they are building a new type of SPRi

platforms where halotag base chemistry could be used in the chloroalkanes and then probably that will have much more robust signals which is what is required for the low abundant proteins for the clinical sample context.

So, this how the technologies you know development takes place. This is one of the approach you can appreciate could be used for high throughput screening which I am sure eventually will come to the market.

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Another thing is which you can think about coupling the surface plasmon resonance technology with the mass spectrometry based platform. Because just imagine that you know SPR having you know its own unique property then mass spec can identify the proteins very effectively. So, if you are just doing a testing of a known protein with a known potential

interactor on SPR, this is what you do usually right. You have a known protein which you mobilize then you are floating one of the potential known protein to find out the interaction.

So, then you are not doing much justice to your actual experiment and the technology because you are already knowing the things so much and then you are saying these 2 are should be binding, but now I am confident they are actually binding this is what you do in a SPR right. What can be actual power of the technologies that you have a unknown protein printed and now you are passing your you know lot of compounds and you know you can see are they binding or not right. And then can I discover something new which is binding, but what is that new thing which is binding which you have no control which you have no idea.

So, then eventually you want to identify that particular binding with the mass spec. So, but you know in real situation if you have done mass spec you would realize you need enough of the peptides which is required for detection of those peptides. And the SPR experiment you are doing these things that you know just very low concentration that is not sufficient for you to give enough peptide for doing the experiments.

So, it is of course, not a novel thought it is just you know a practical concept which we want to employ to really find out the unknown interactors, but to detect them using mass spec, but even the practical concept can become very difficult when you do the actual experiments.

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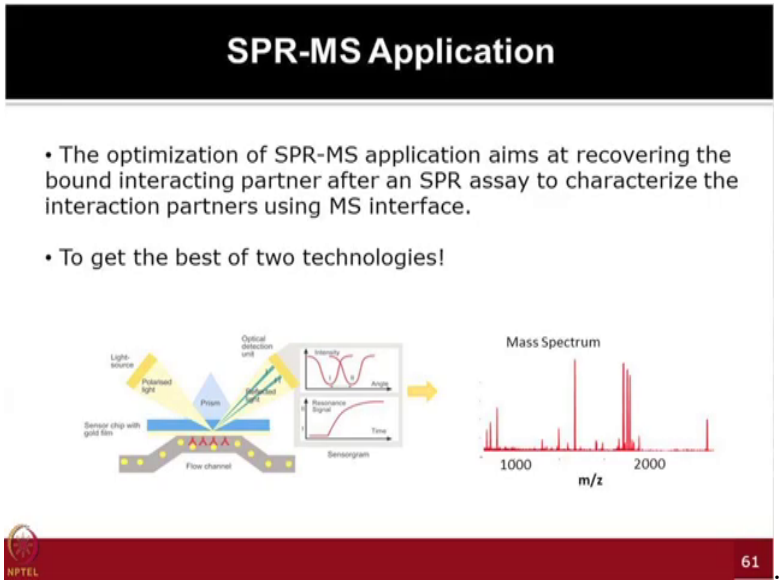


So, the concepts are you know very straightforward. We are employing one of the SPR technology and one of the mass spec you know technology; two together are trying to get the best of the two technologies to identify their known interactors.

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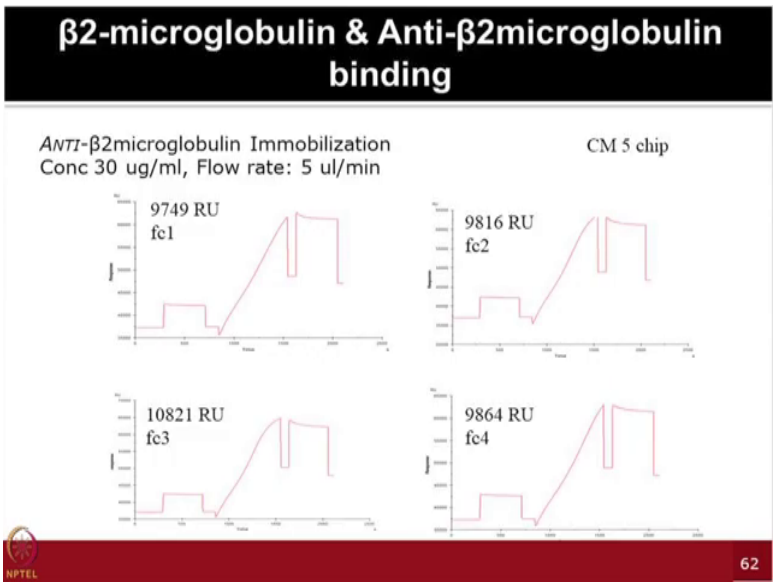


In this case again we started as a proof of concept which are 2 microglobulin experiment and then use all the 4 flow cell because you need lot of analyte to bind which you can recover and then use for the mass spec testing. So, all the 4 flow cells were immobilized.

And now when you are passing your beta microglobulin protein then you are enriching them and we actually you know use it pretty high concentration 30 micron per ml flow rate was used 5 micro per minute all the flow 4 flow cells were employed

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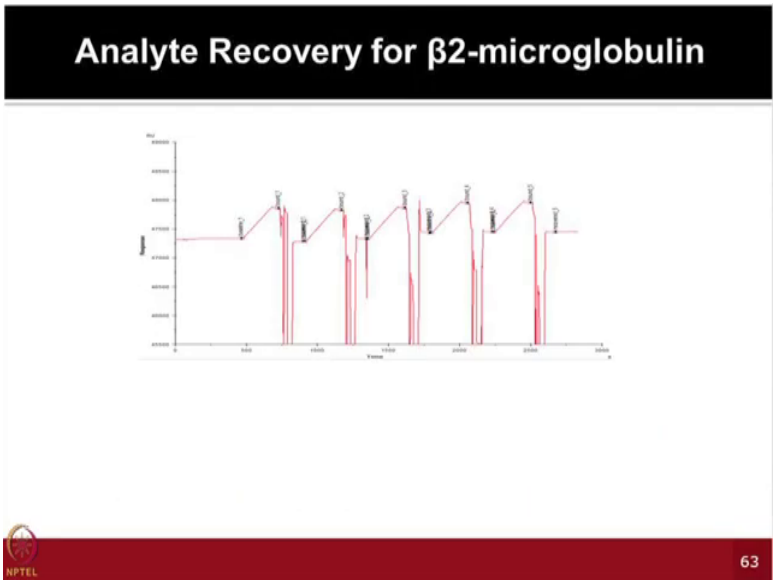


After doing you know series of cycles, so, we did that maybe almost 20 hours or so, several rounds on all the 4 flow cells, with the intention that you know enough of the protein should bind beta microglobulin should bind on the anti beta microglobulin. And then can we chop of that particular bound protein beta microglobulin then get peptide extracted out of those proteins and then finally, we can detect them using mass spec that was the objective.

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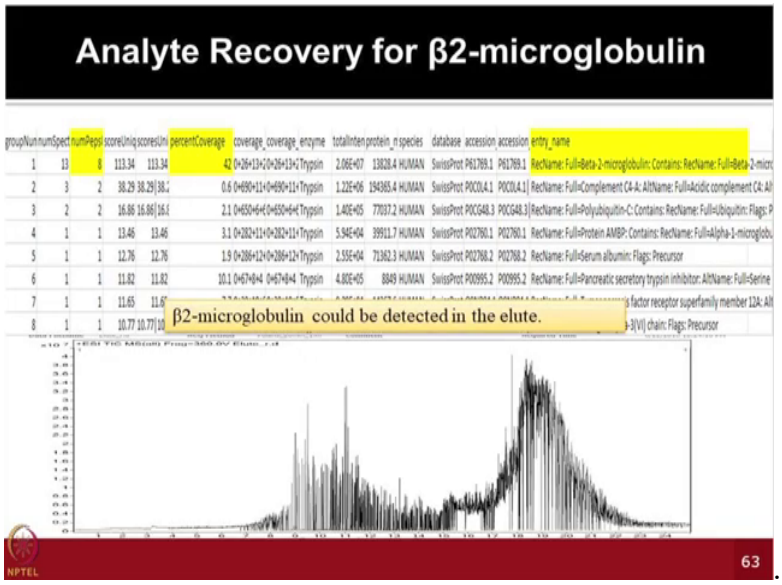


So, after doing mass spec success was not so great but it still we could see it; peptides coming out of the same protein of interest at the top hit; beta 2 microglobulin you can see here as a first hit of mass spec and this is the kind of chromatogram which we saw.

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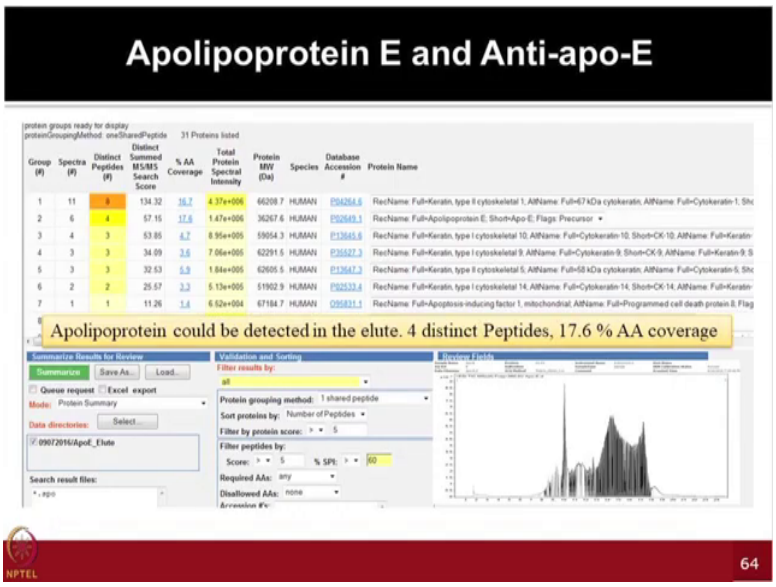


So, then we tried on another protein which was of the physiological interest apolipoprotein E and intention was again to you know look for their binding this time we could see 4 distinct peptides. So, at least you know good news was and this one we are using (Refer Time: 30:45) platforms not the latest (Refer Time: 30:47) technologies.

So, we could see the right protein and detect the right peptides which was good news, but of course, many times you do not have opportunity to run all 4 flow cells for so many round of cycles to really enrich your proteins. So, how low we can go is what we have to now optimize it using mass spec based platforms, but this can be something which is useful for biological experiment when you really want to identify the unknown interactors we receive binding, but you do not know the protein of interest. So, you can employ both SPR and MS platforms alright.

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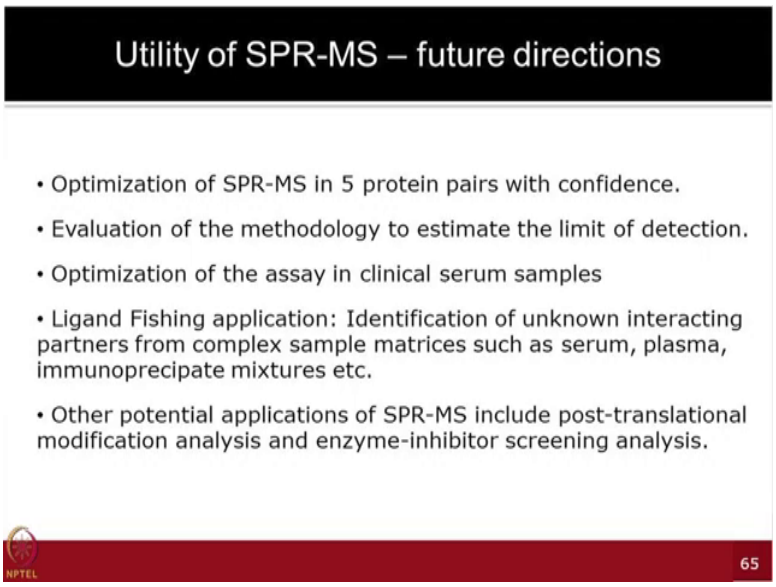


So, SPS MS you know now we have at least tested as 5 protein pairs which looks more confident we are kind of evaluating those.

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Technology to see limit of detection what could be best used and some of these could be you know heavily useful for the clinical assays especially serum type of samples, you have a known protein of interest and you want to see you know where it binds with other interactors. So, a lot of ligand fishing applications can be you know thought of based on this application; which is what we are testing out and you know some of the GE application scientists are also working with this.

So, this particular technology having lots of applications including it could be used for the PTM kind of analysis as well as the enzyme inhibitor screening analysis, but you know now we have to really use the best of the mass spec power to go to the lowest detection limit with the minimal run cycle with the minimal repeat cycle then only I think this can make some impact in the actual samples of interest alright.

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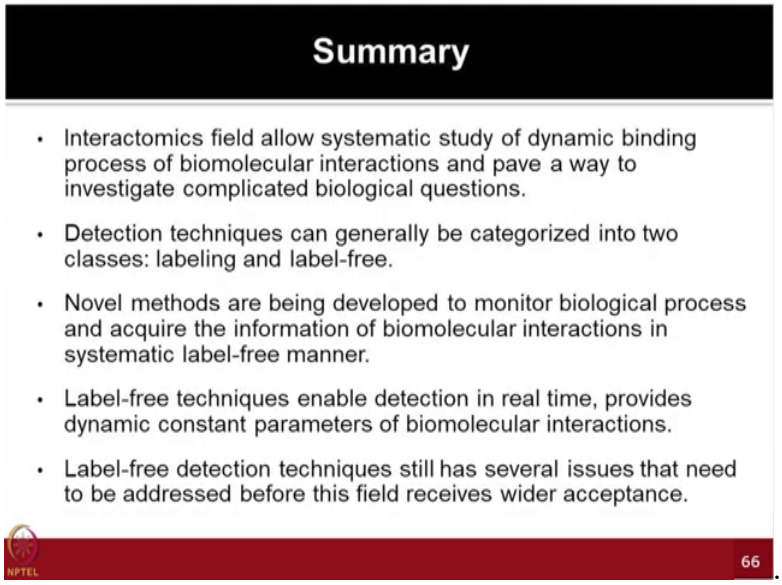


So, in summary I think I have given you probably a broad overview of various interactomic field you know in which way the dynamic molecules, to really understand them you need a gamut of technologies, you cannot just rely on one that I have studied in this course and I can now start going using for review of my application. You have to very open to look for various type of platforms which are available to you. I am sure with overall you are probably

convinced that you know there is lot of potential of using these technologies; label free technologies.

And the different platforms available starting from you know looking at conductance based principal, reflectance base principles, interference based principles; many of those could be utilized you can choose which platform can be useful for your kind of application. But you know I think you have to be really open to think about what should be your experimental strategy starting from where you should start from discovery workflow to reaching to the validation phase and then for you know finally, you want to translate those finding to the you know actual products.

So, you have to follow you know a good pipeline which involves very much you know all kind of technology which you are studying in this course as well as which are being taught in the other courses in the proteomics field right now.

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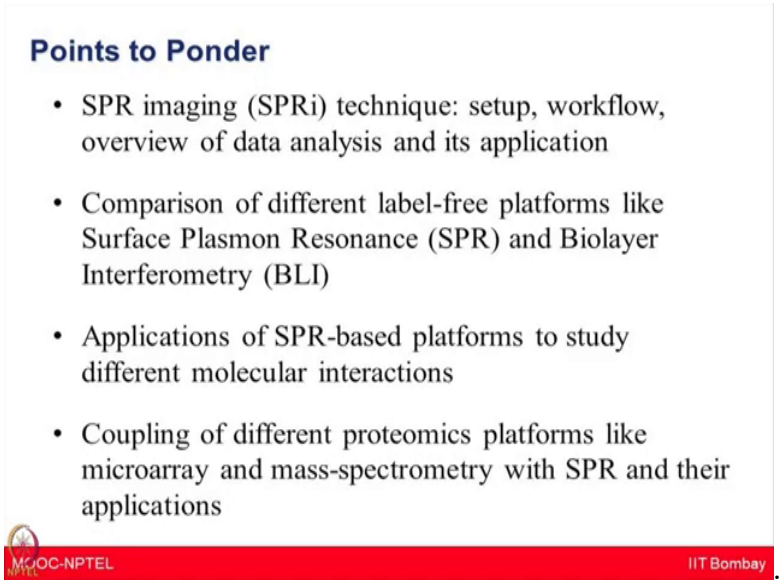


I hope now you are able to understand and appreciate the utility of label free technologies, in the field of interactomics and also integration of novel technology platforms. Especially SPR imaging, SPR with mass spectrometers as well as other technology platforms in the proteomics areas; which could be utilized for multiplexing for these interaction studies. That can even lead to identification of novel interacting partners or establishing with confidence the high throughput screening for the drugs in a given context.

The experiments that were discussed in this lecture would have also provided you an insight into wide applications of label free techniques; it will definitely encourage you to think about how to plan these experiments using label free biosensors especially for your own experiments.

And I must say whether you are going to work in the areas of proteomics in depth or not, but you will at some stage need to study protein-protein interactions or protein drug interactions. In the upcoming lectures we will also discuss about coupling of immunoprecipitation with mass spectrometry to detect and identify the interacting partners and the molecules of your interest.

I hope these novel technology platforms which we are discussing in the last 2 lectures and in the upcoming lecture will make you much more aware of available technology platforms for your applications.

Thank you.