Lecture 23: An overview of label-free technologies-II

Welcome to MOOC codes, on applications of Interactomics, using Genomics and Proteomics technologies. 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's 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, two major technology platforms, for label-free biosensors like SPR and biolayer interferometry. So, my today's lecture, I hope is going to further, unfold the applications of label-free technologies, in analyzing different molecular interactions. Let us continue, with my lecture, which I had delivered, in this workshop. SPRi, SPR imaging, is one of the high spatial resolution platform, which allows for high-throughput analysis of various type of bimolecular binding,

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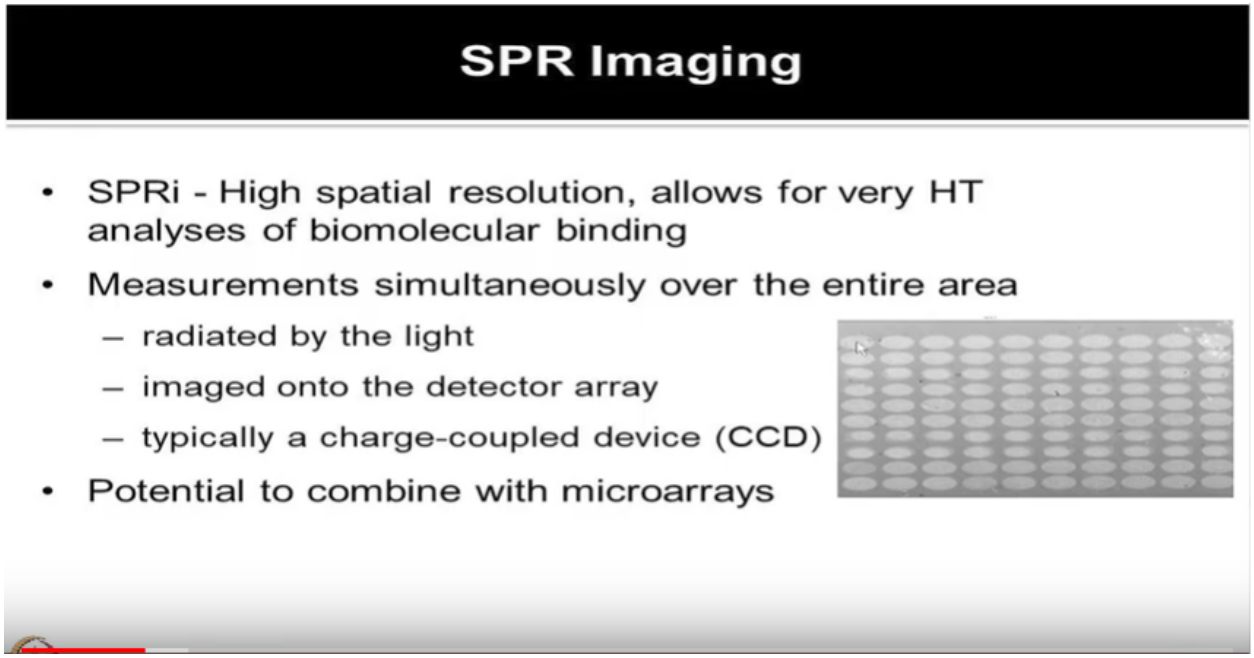

interaction that you want to measure, here you can, intentionally, that you are using imaging platform. So, imagine that you had the gold chip. which was your using for a SPRi, but in SPRi gold chips, you have the very small region, where the gold part is there, rather if you have the full chip which 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’s not actually microarray. So, that kind of concept was used can we image the whole chip, rather than you know exciting on a specific angle only, which what you use in the SPR case. So, you a reradiating 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 technologies because anything now, which you can do in height so, put a way, for the whole chip

it's very much compatible, with the microarrays. So, this is where, you know one of the image I'm showing, with one of the experiment, I was doing in Josh lab earlier, when we, we redeveloping 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's not the actual, you know you're not building fluorescence, these are some of the pseudo image, obtained from this kind of platforms.

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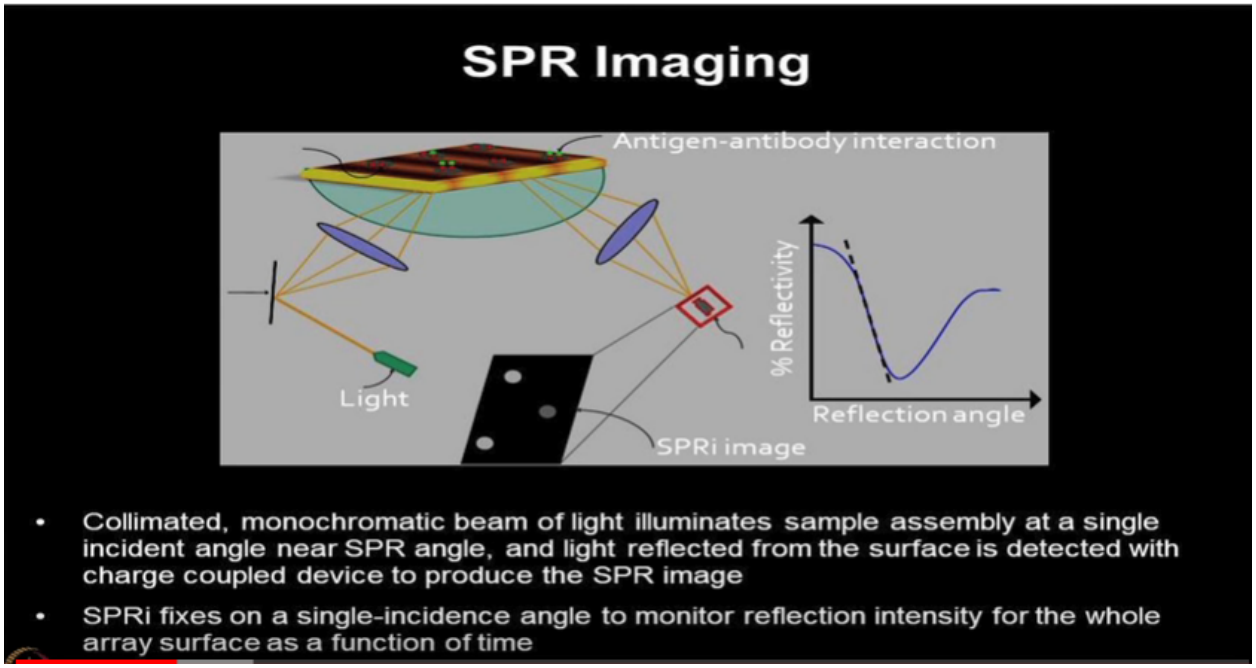

Which shows you compatibility with SPR imaging so, what happens here conceptually, we have this prism, we have this gold chip, but nowhere we are exploring, the full chip potential, to scan the whole surface here and then by generating this SPR image, again the concept is same we are still looking for 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 this SPR experiment and that's our you have to play with the conditions, to find out what can be that best angle, which can be fixed for the whole chip surface, to do the cleanup versus, once you find that and again that may not be the best and most accurate angle, it's a nineteen you have decided or twenty or decided you may have to play with more, to find out what can be the best angle to, 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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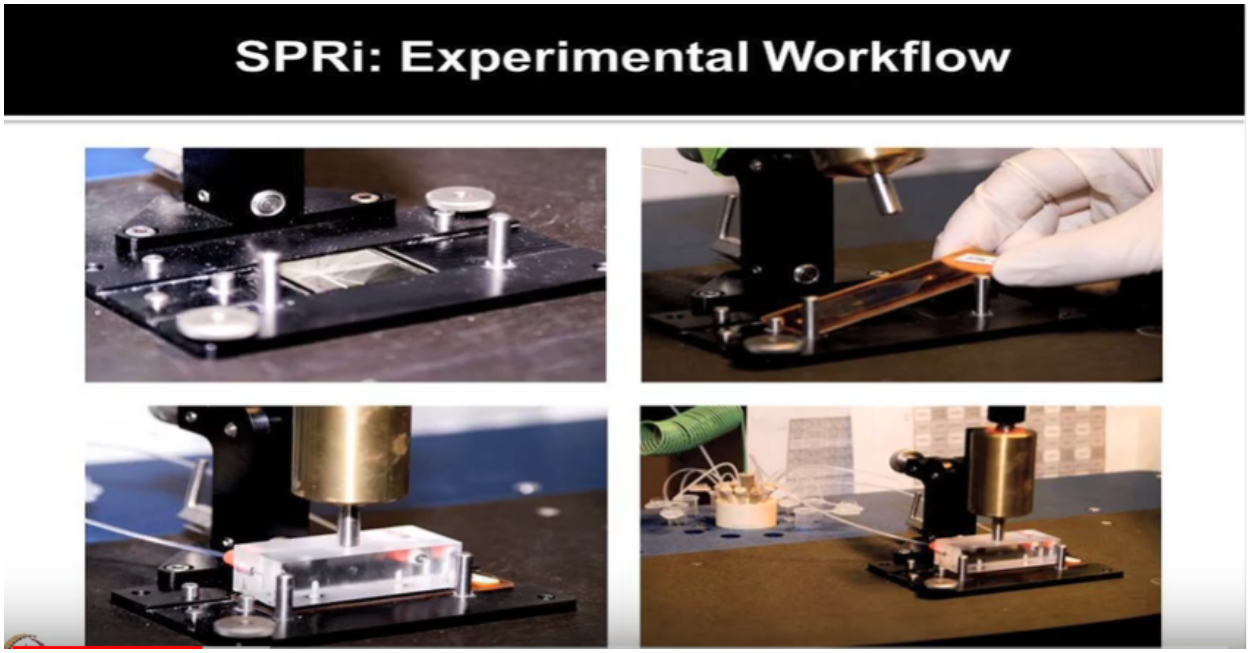

I’m showing you not one of the workflow, which was done several years ago, which was on a prototype, to give you feel of you know how these kind experiments can be done. So, this is you know prism here and this is a chip full of the micro really, kind of chip, where you can add all the you know protein 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 this peer kind of setup and it's you know just reminds probably Josh, that you knows one of the old setups we have the prototypes ,where now you can test a tenta body's, for example or a 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 is going back, in the washing containers. So, these kinds of platforms could be used.

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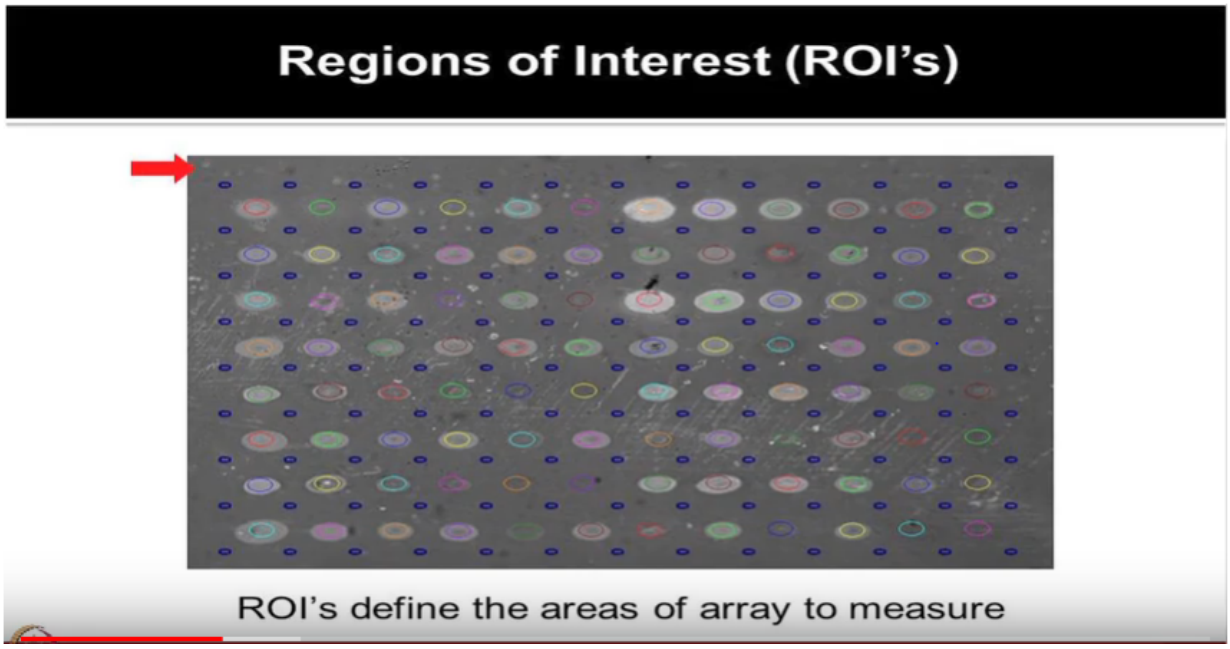

And it result into these images, which are the pseudo images, as I mentioned to you, it shows you these kind of spots. Now all these red green, what is shown here these are actual spots, but along with them whatever you see the gray color, is the widgets background. So, software are artificially, add some blue color dots, these spots could be used for subtracting the background. So, in this way, now your you are ready to test lot of molecules, unlike the for what you can do? Just by using the listing technologies and these are known as the regions of interests, are wise in any kind of microarray experiment, any kind of a superior 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 Intel these spots in combination with the background, what is there?

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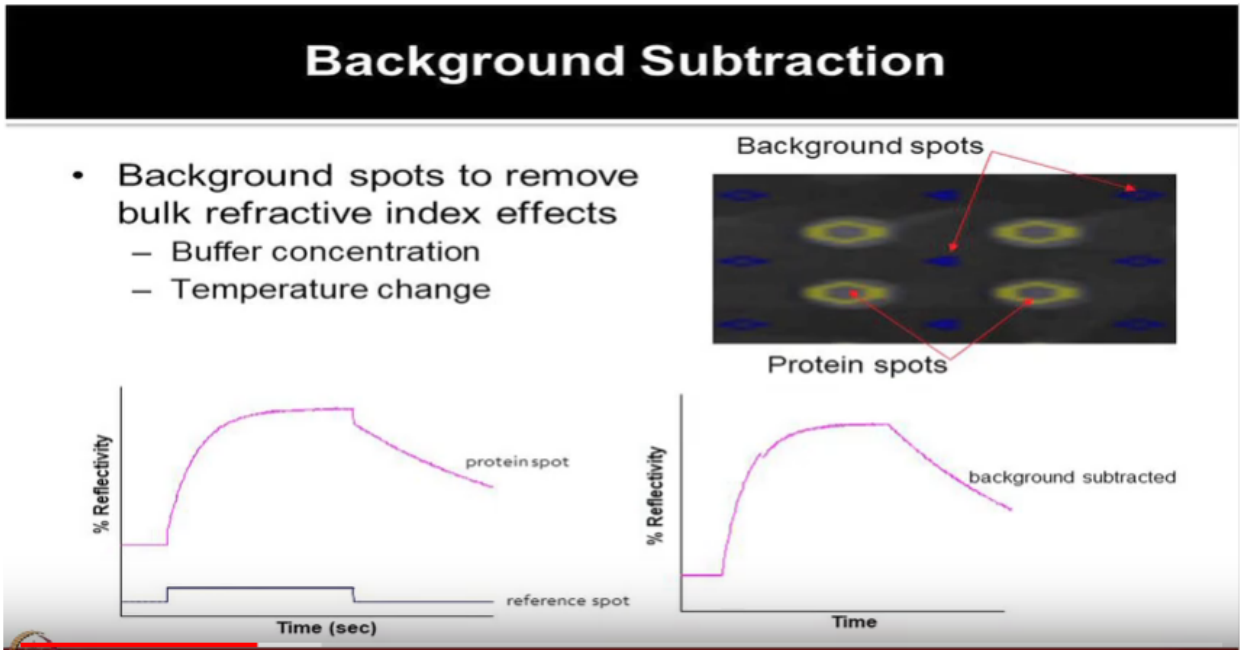

So, therefore for each of these spots, we had four of the different spots. So, if you subtract the average of these four, from this particular one and same you do for every spot then probably, you're only measuring, what is printed on each of the features. Right? Then you have removed, all kind of side 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 chip got senior sensor gram looks little different down. Right? So, these are the kind of thing, which can make the your curves very different, if you're just not using normalization or if you're using those regular subtraction, which is very crucial.

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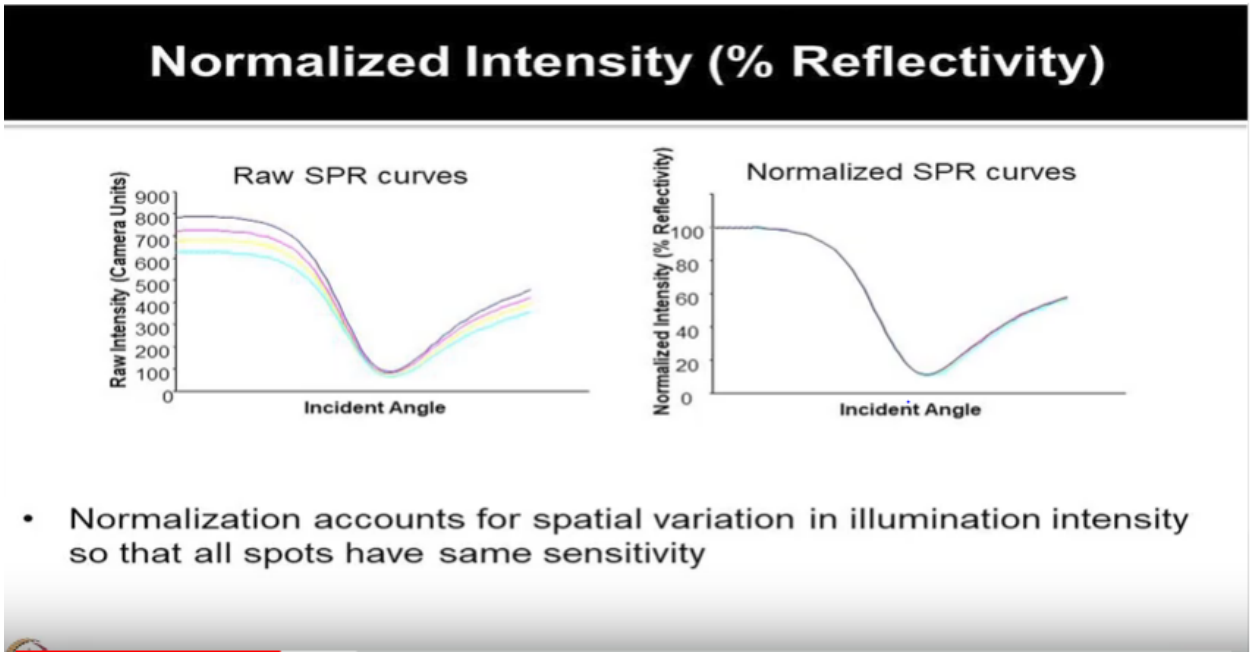

Why should really normalize these features on the chip surface then it becomes, more critical here, because you have, many spots printed on the long chip and imagine that you know when the liquid is flowing, from one side and reaching to the other side, it will take some time and your you know you are 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 SPR curves and everything, is now looking same then, now you have a pertinente compare, their signals. So, the SPR signals, which is we talked about sensor drum, they are the one actually if your I is I either pretty expert, in tune to look at the data, immediately start telling you lot of information, SPR data, just you know it shows you the different curves,

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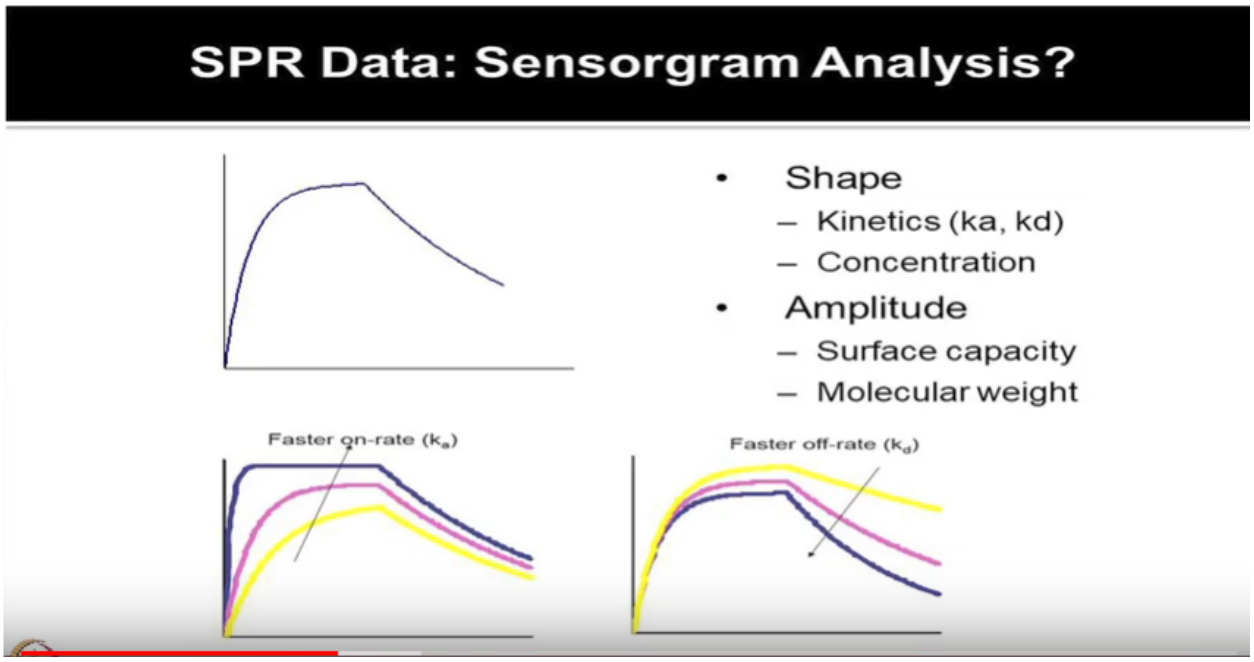

will tell you that you know, what is happening? For the kinetics how much concentration, I can see for those proteins what is this apace capacity molecular weight etc and many times you know these kind of curves, which are showing, you the just the difference, in the on rate and off rate can be very powerful, because imagine that you know you are working, toward developing different, drugs and objective for you know, their those drug development probably, I would have quoted several examples, if you want to really make that drug work very fast. Right? You want immediate effects, for you know any accidental and any kind of no headache kind of things, you want just very quick response. So, you're on rate you are looking at, 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. Okay? Let it go off slowly, no you do not want it to just you know show a 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 date and offer it could be different and those can be so, detrimental or useful, for your kind of drug discovery you know portfolio, which you want to develop. So, therefore these kind of lockers are very crucial, as you're watching and there are many companies, who 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 bio similar 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 since program, 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 a lot a visual feel, of doing those analysis.

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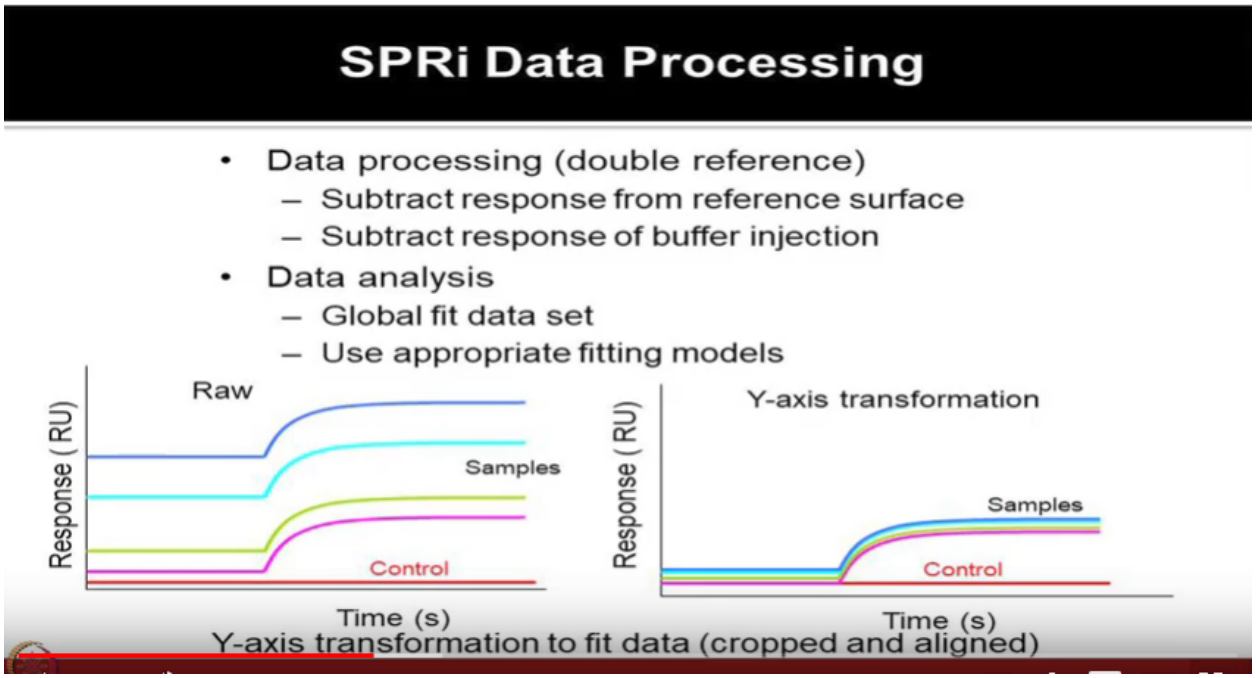

But as I said, you know if you want to really get the proper values, you have to do good cropping you have to do good fitting, in this case for example as I mentioned, your response timing is different because, you have different molecules printed, on the whole gold surface where you have some starting. Right? Now and some starting later part so, then your response units are shown differently here, but now you a renormalizing all of them and then you want to see comparison of their responses, throughout the whole experiment. So, therefore data processing becomes very crucial.

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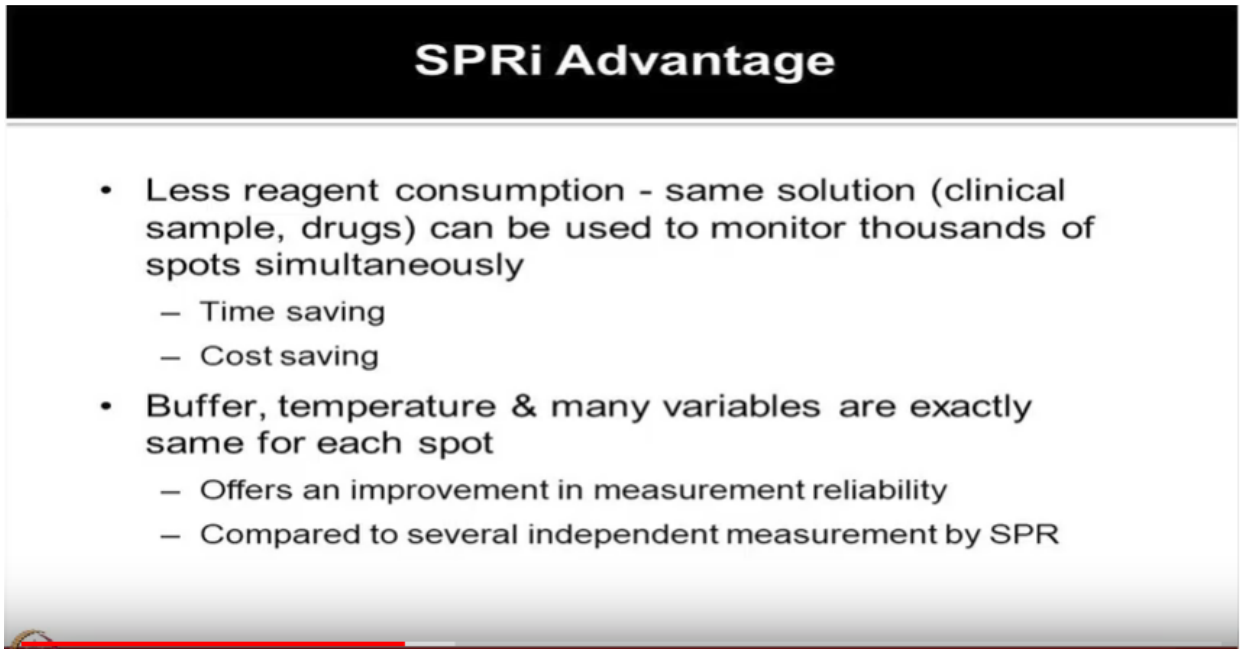

But advantage of using SPR imaging, is like high density microarrays where you are using less of the analytes, that you want to test in the same kind of sample, which you have available especially the drug, molecules which are very low in quantity or some other clinical samples, I think you have opportunity over there, to now test out on hundreds of features simultaneously. Right? With SPR imaging platforms so, that is something you know 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 or things, even you are doing under controlled condition, but if you split hundred analytes, testing on twenty five chips, what's is doing on one, will have huge difference. So, buffered 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 way of having the controls, to negate those kind of you know negative effects from, coming from those molecules. Alright?

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It's so, latest popular technologies one of them is,

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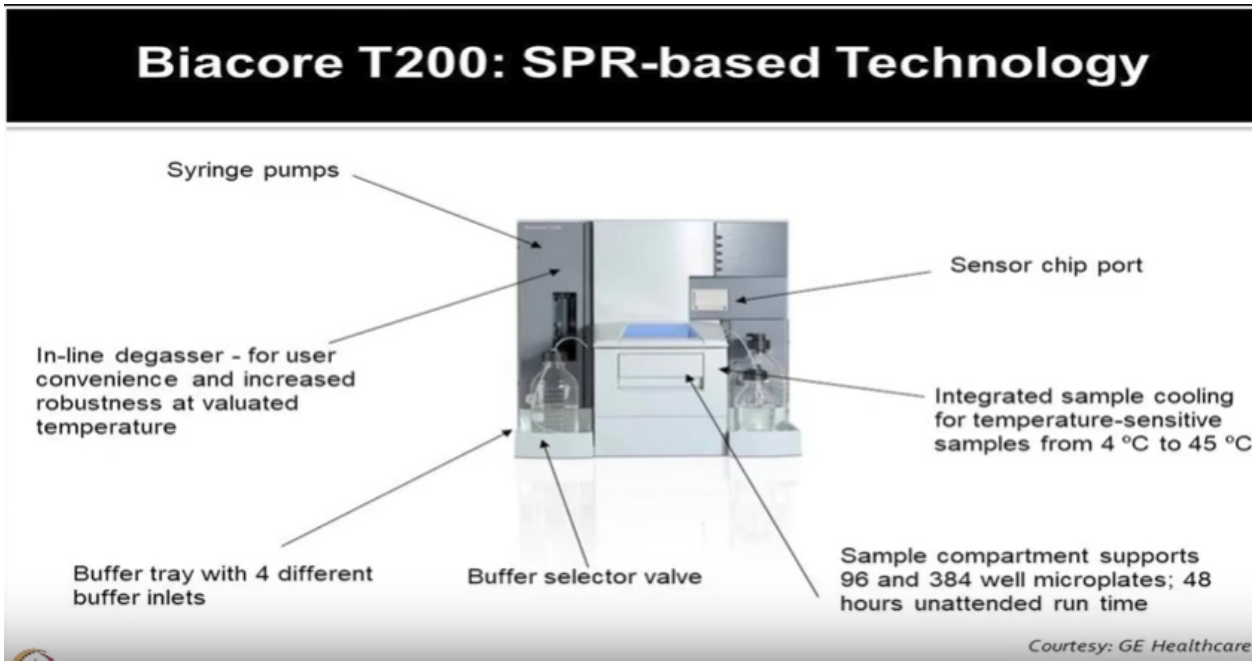

Biacore t200 is one among them, offered by G health care.

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There are many type of chips which are coming, from these particular type of technologies, where you know how you want to immobilize your molecules, sometime you have different type of car boxy methyl l based surfaces, sometimes nickel nta kind of shift 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, bia code t200 is. Right? Now one of the industry leading technologies, which is pretty much GLP approved. So, whatever you obtain here, can be very much bit same for every or other kind of approvals and that's a 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're very costly you know considering, the how much cost it takes to do these experiments. So, many times if you are in the discovery development, mode and you have hundreds of you know compounds or libraries to stream, this may not be the best platform to start, with because you are limited, with the know how much cost you have, to screen that when you large number of compounds.

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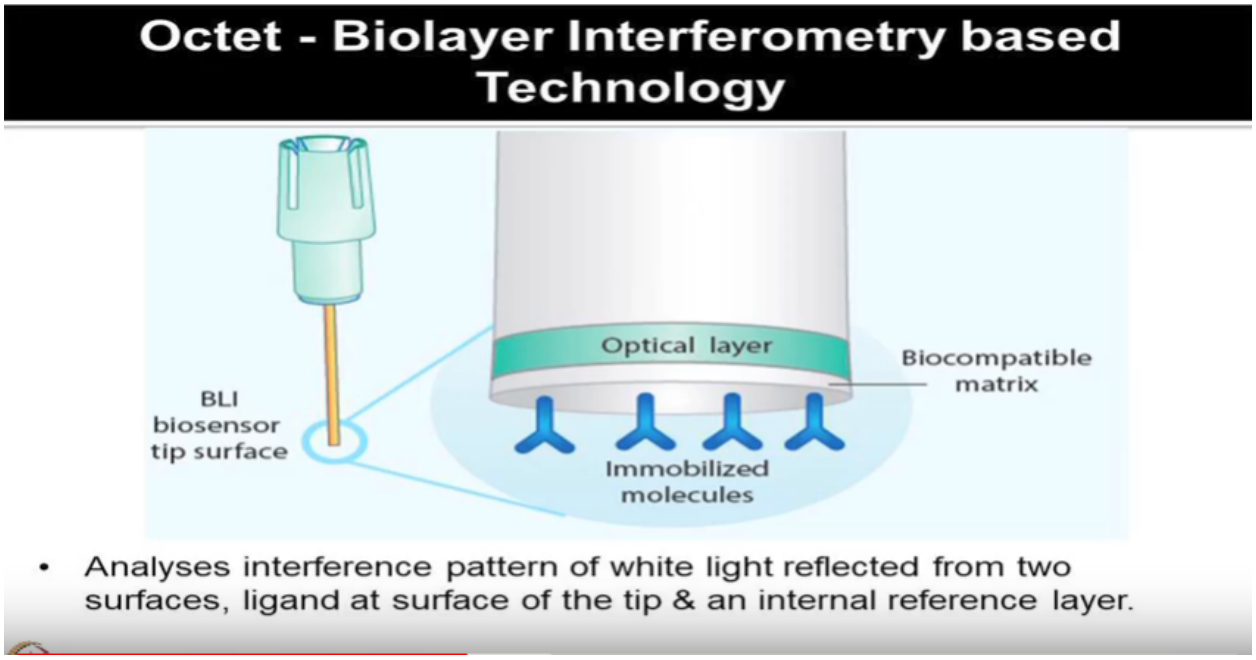

So, new technology platforms, like one which I'm showing you here, which is bio layer interferometry, based platform can be very powerful, for first round of screening, it may not have that sensitive, on date off rate and those kind of KD values, which you want to obtain, but it will definitely tell you binding, happening yes know, in a very quick format using your solutions, what you have for different type of molecule so, test out and here they are looking at the interference pattern. So, on this sensor, there are some these antibodies, which you want to test out the removal eyes and then they go inside, they like Eliza plate and then they those sensors are going to go in and test out your, your binding using, the interference chain which is going to happen. So, this something is we have a follow-up lecture. So, I'm 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's say you know 200molecule, which is of your interest now, you can go to BLI to narrow it down, further now you can use probably a code to test out you know how many of those you want to no matter the exact rounded off rate. Right? Okay? So, there are many label free, platforms which are possible I'm restricting, that it's now the applications of those mainly built on their SPR. Right? Now because that's what something which we have currently, available at I it Bombay and I'm also running a code facility, which is offering services for any type of applications, for internal users and external users.

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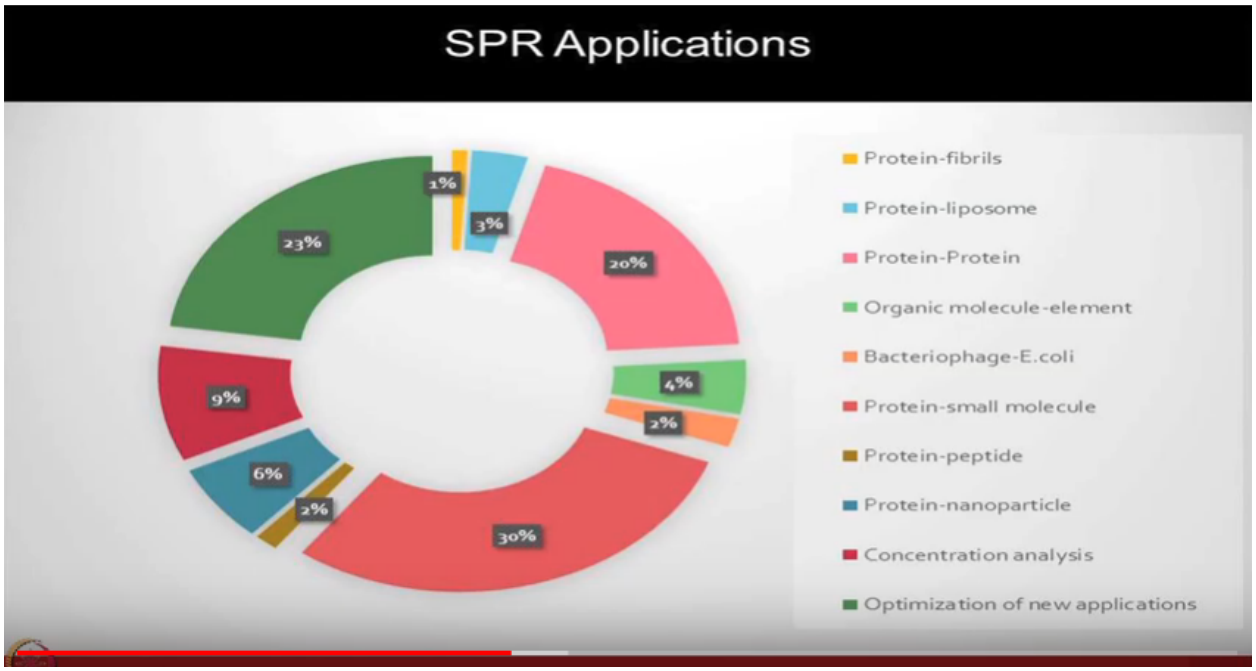

And the kind of you know this particular distribution, but what it shows to, you that we are doing all kind of applications here, looking at even from protein fiber interactions, protein iposome, protein, protein various organic molecules and one we know interesting experiment came when somebody wanted to print bacteriophage virus, on the chip and this with the e coli way that those are 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 nano particles, concentration analysis something, which we developed a new assay and optimization of many new applications. So, that's a the ongoing thing, we always want to see that what more we can obtain from these instrument platforms.

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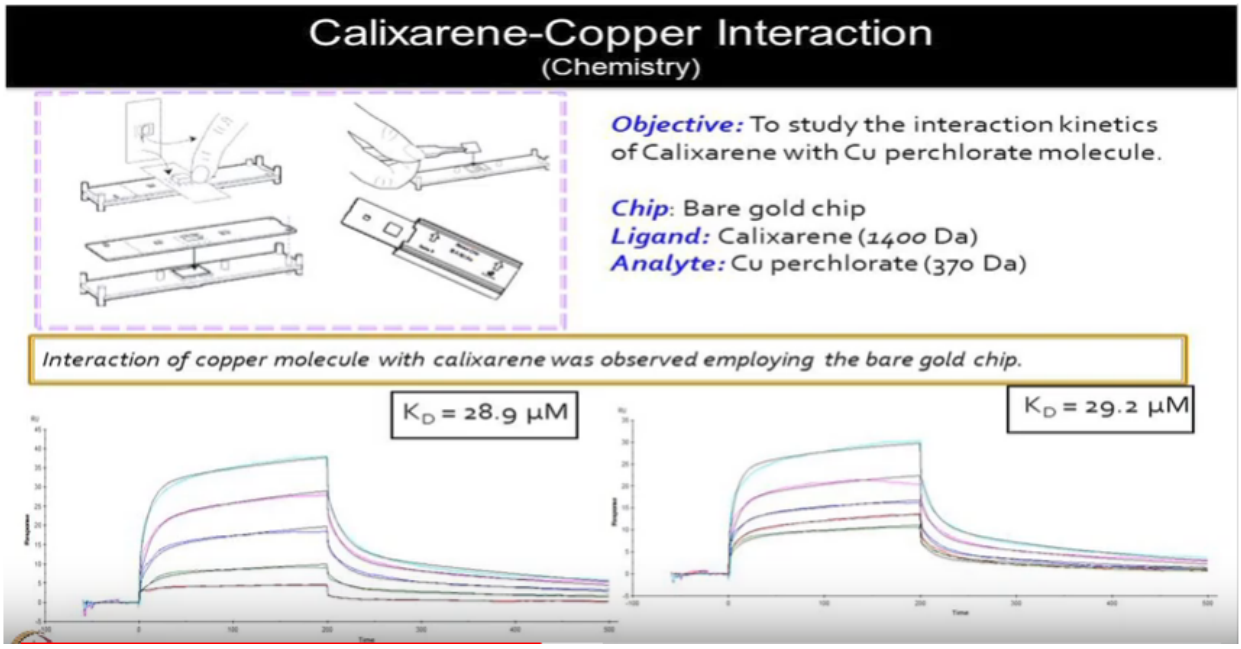

So, I'm now kind of you know flashing it through a couple of application slides, with just an intention, that what all things can be done, on these platforms, these are not my results, just without coming from our facility, whichever people are running the experiments, but what it shows to you that you know variety, of things can be done, for example one of the chemistry faculty lab, was looking at one of the calyxarene 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 themselves, of those and after doing those particularly, this is a step which are shown to you. So, sometime you may not have ready-made chips available, for the commune of function action which you want to follow and there you can use the bear chip, where you can try out your own, immolation chemistry, which could be then used to further do this SPR experiment, don't be a code chip. So, that's what we have some bare gold chips, as well. So, this one showing you two different experiments the KD values obtained, are pretty close and that was at least you know interesting experiment, for us to try out, because it was not the ready-made chip available, for it and if still immobilization was pretty reproducible.

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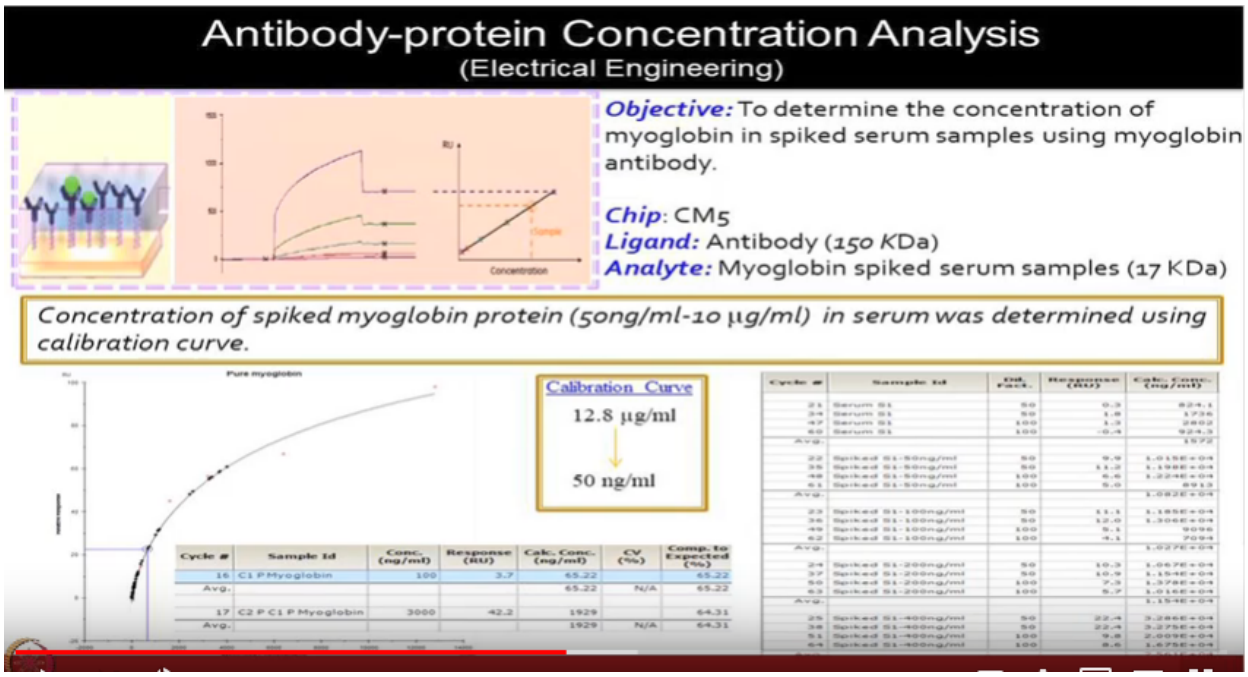

Another from electrical engineer, their intention is to have some benchmarks. So, let's say you know, we're core technology the benchmark, available in the, in the field, if you can measure you know an interaction, and generate some KD from biacore and now if you develop another bio sensor and you can 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're 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's 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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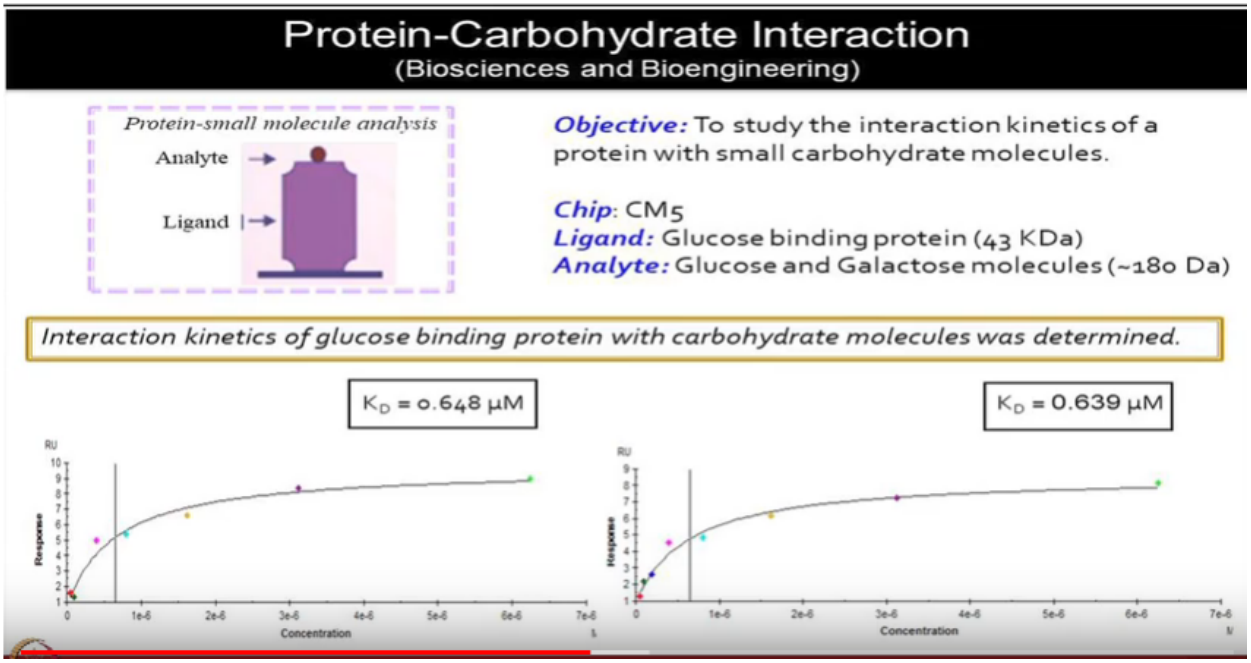

Another experiment test from the bio labs here, in the protein carbohydrate interaction, which is another very common thing which we see, many people want to look at local binding protein, a different type of carbohydrates, binding to the proteins and again I am showing you duplicates, because it's become very crucial, to give us the confidence, the different experiments, are giving the same KD values, again I'm not talking the biology of it, probably I should not talk isothere's work, but I'm just giving you the feel of variety of applications, which are possible by doing SPR kind of technologies.

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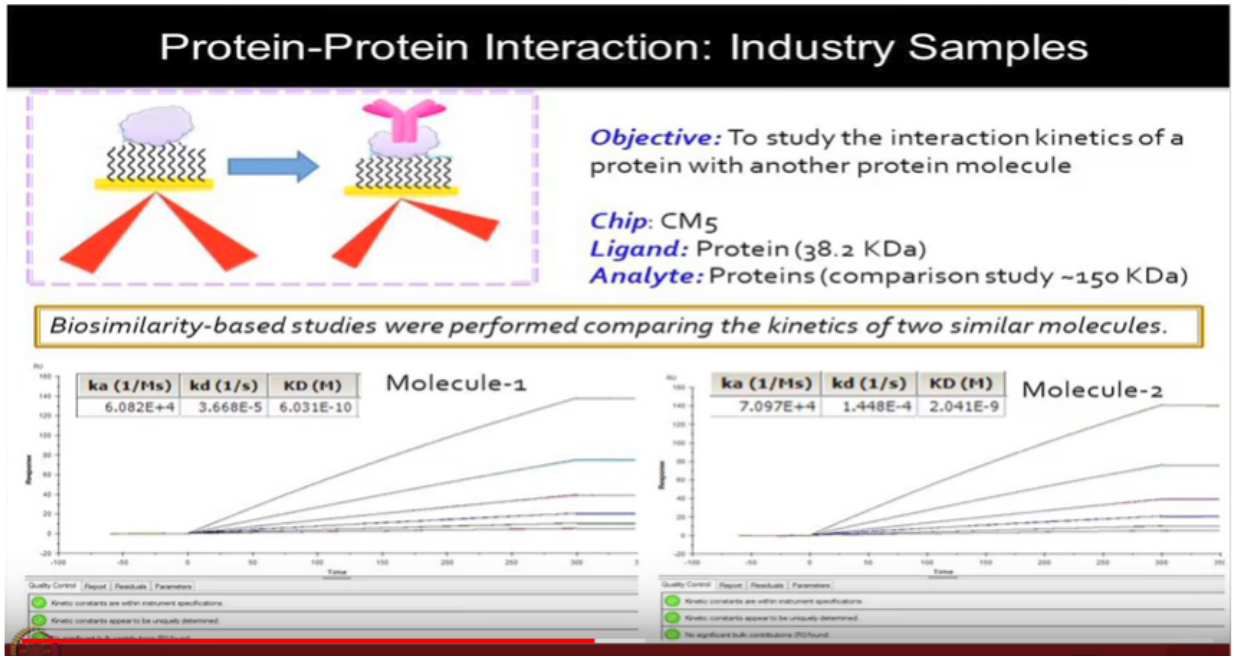

Now one of the industry sample, which of course I could not disclose, which was trying to compare, that what data we obtained on bia core, how would that you know similar or d is similar, when they are running to their parent company somewhere in Sujarland, and they had that kind of numbers and the you know KD is in their mind and they wanted to compare the things, here and those molecules showed pretty close, binding based on these vehicle experiments.

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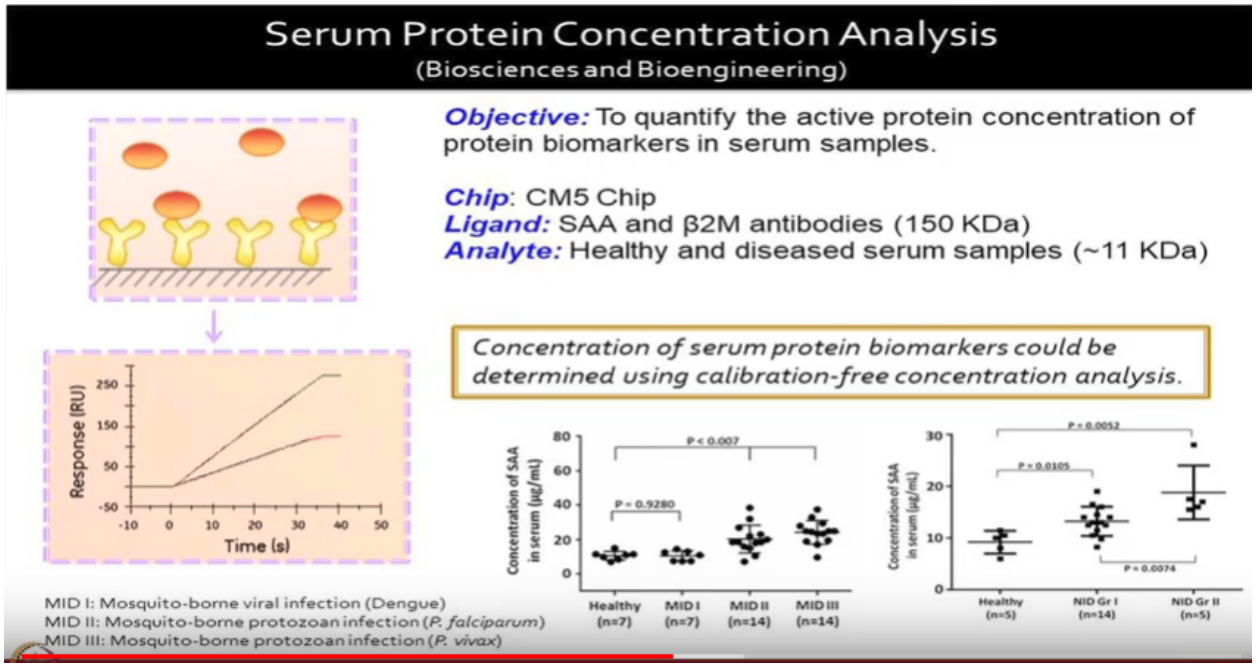

We also have some faculty from chemistry, who are looking at protein a small organic compound, their interactions, in this case one of the unknown perimetry is not known to us7k d protein, was immobilized and then, we are looking at how small organic compounds, can bind to them. So, again many of these times the KD's are very close and that is giving us you know good confidence, that these experiments are induplicate or working well, on 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 dye agent quiet track based platforms couple, of protein we identified which looks good to us and the potential biomarkers. So, we thought can we measure, the level of those proteins, in this 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 a CFCA, analysis where you know different type of in you know patients, from this is one of the malaria project, when we had patients suffering, from either Philippe de Mar Y Beck's malaria or dengue fever and we wanted to measure the level, of one of the proteins, serum iodide aid and we wanted to see that, you know how that protein concentration, is different in different type of conditions and because you already had idea, for this protein from our other data said discovery track dataset

So, we, we felt pretty confident that you know we can now measure and this is what you need you know you cannot do, validation on a large number of samples using mass spec or other type of technologies. So, then you have to come down to either Eliezer 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, to show that you know we can actually measure the concentration, of this protein of interest to us, again you know some time you have to develop these are seen in the beginning. So, you need pure protein it's good idea, to try out something if you are planning, to do this can experiment, with the known proteins, for which you have the purified proteins available, build the confidence and the sea conditions from those then you can apply for some unknown compounds as well. Alright?

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So, I'm not showing you some data, actual data, from two different types of technology platforms.

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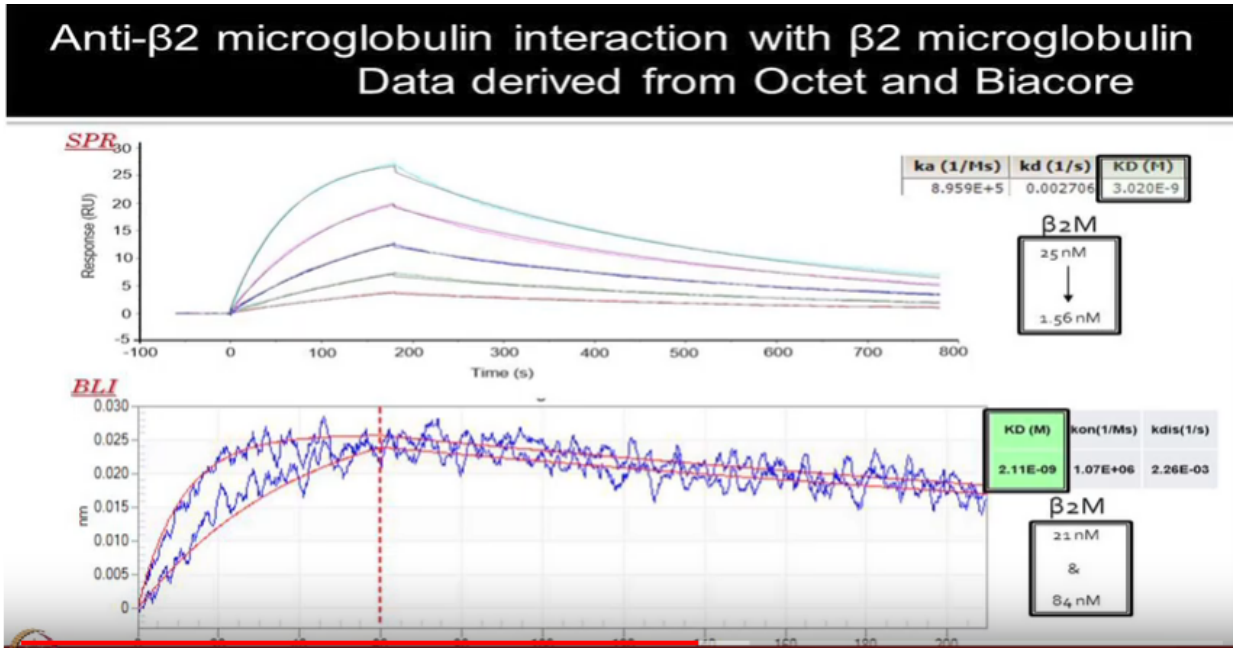

So, we compare for a couple of proteins both, BLI platform and Biacore t200 platform for few experiments, because you know the cost wise they are very different, but sometimes the you know looking at the acceptability, wise things are different and the kind of GLP compliance, was only one platform is more compatible. So, can we use this you know at least the VLI platform, as the first level of screen, with high confidence. So, in this case we use anti B2 microglobulin and the scent about a and the protein interaction. So, using SPR and BLI we tried binding, for various time points and acid which we did different concentration series, came pretty close actually you know, this is showing you 20the overall KD is almost 3, e to the power of minus 9 and it is also 2 point1 1 e to power minus 0 9, these things just imagine, was done just you know in alike this kind of instrument what is being brought here not available in my lab that time. So, not in the best optimized conditions, but it's still, the way I can see there's not huge variation, just the measuring this kind of you know the abundant molecule bindings.

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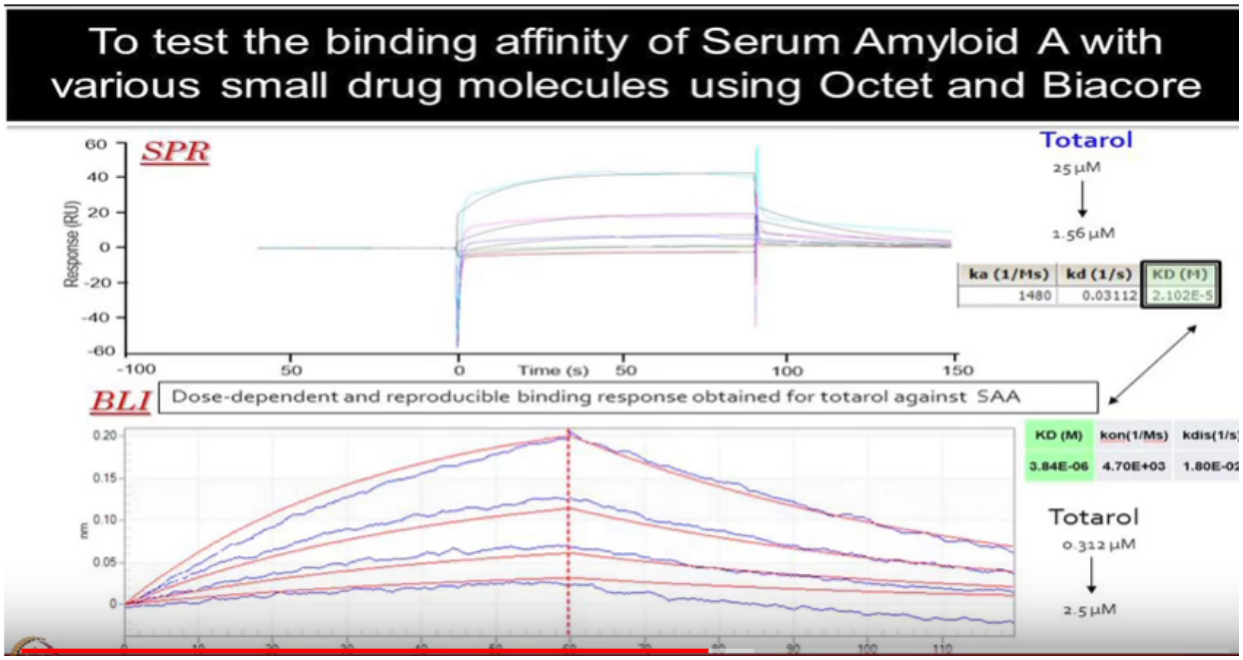

Then we looked at some of the, you know compounds of interest, for a given project, when we had some drug, put it all to look for their binding and again things are pretty close, you know coming from the two platforms available.

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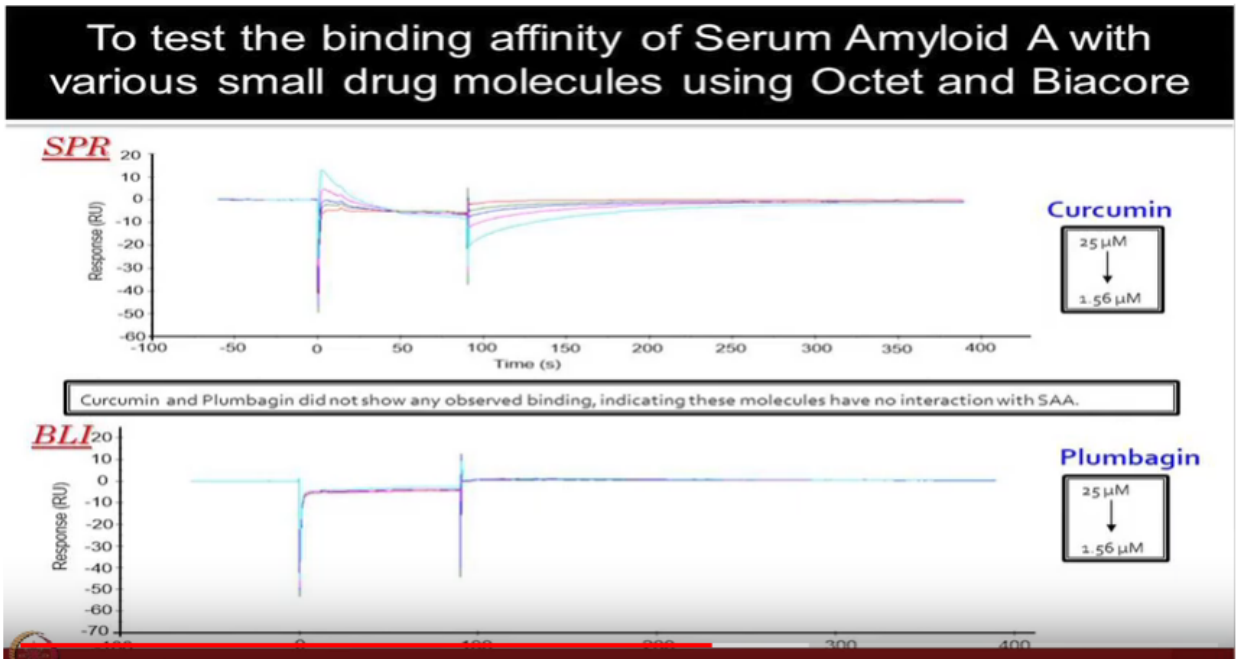

We also tested you know another, drug which is shown here cur cumin and from come again which we are testing for some bacterial protein, against those and we are seeing you know, the changes that are pretty, close coming from both the platforms. so, you know what I want to convey you that you know, many times just don't 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, it start you know using those platforms, which can narrow down your, your leads and hits, then do the final experiments, on something which is really a 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 some time you know a good experiment may involve, every technology which we are you know studying in different workshops. Right? Now here, it starting from you know discovery from my mass spec based of and microarray based, of you know take those leads forward and then validate using, this 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 that there is so much need for having, integration of new technologies anti development, of new technologies and there is a coupling, different existing platform, becomes very crucial, in Josh lab.

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We thought couple of years ago, and many people are still working, on developing those technologies of coupling, an aqua tech no logy with SPR imaging based platforms.

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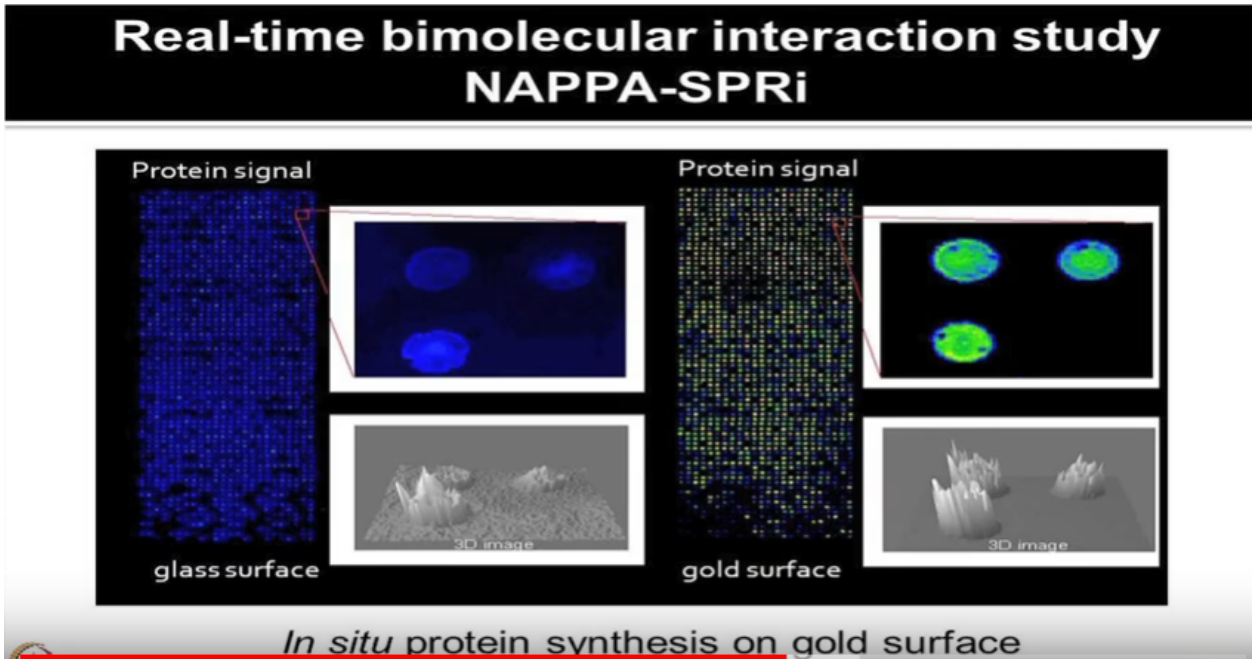

The initial experiments done, by Emmanuel tests and then I joined the lab we're actually you know these are quite old Napa's chips, 2006-2008 that kind of timing. So, the glass slides are showing these kind of signals, which I'm sure by now you'd have seen it has improved tremendously and then the same features, printed on the gold slide, are showing much better much more intense signals. So, first of all the objective, was to see whether Napa concept can work on gold or not, because so, far is all done, on the and 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 3d views an AFM images, it was I think you know apparent that probably, Napa could work on the gold surface as well, but the traditional NAPPA chemistry, which you are aware of having you know many components in a master mix. Right? Which included, you know BS a BS three cross linker you had the capture antibody and of course your clone of interest, the cDNA of that having GST tag. 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.

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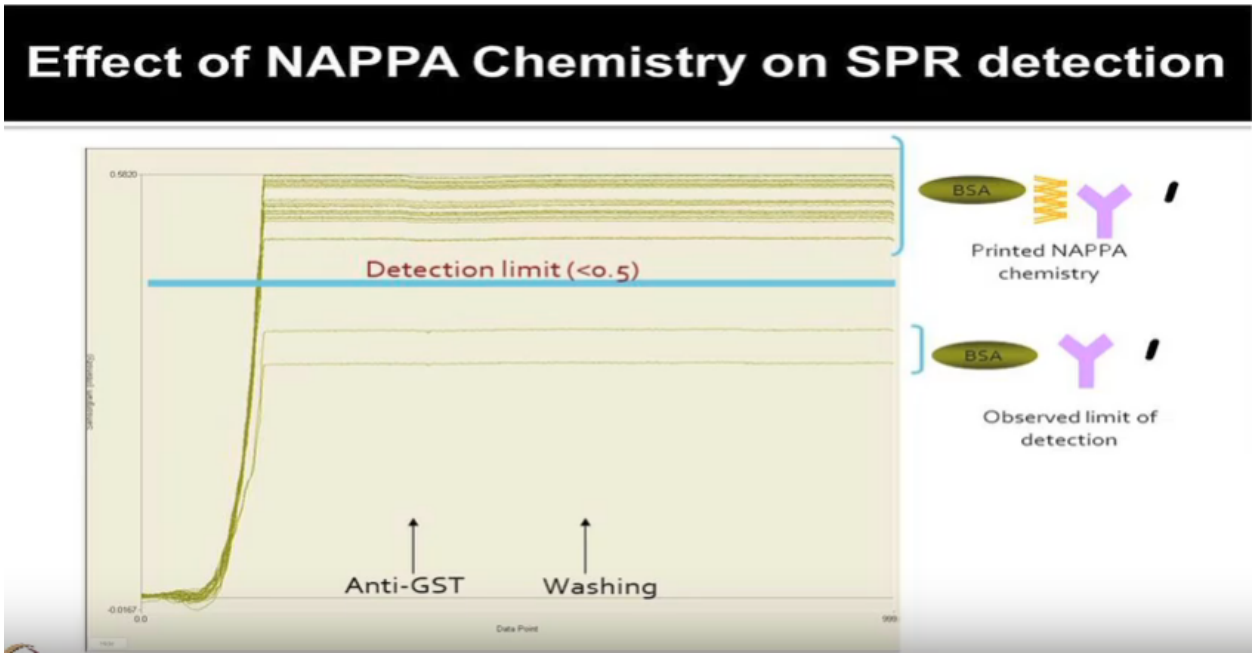

The baseline, when you're printing so, much mass on the gold chip and in this SPR kind of platforms, you're only measuring, that how much, binding you can see happening. Right? So, to begin with itself for the baseline itself is going off the response unit. So, here you have literally no room, to find out how much binding you can observe later on, because with the buffer it alone, the base in 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 an opera arrays, think about the combining the two, two hardness the both the power. So, if you add the entire gist 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's 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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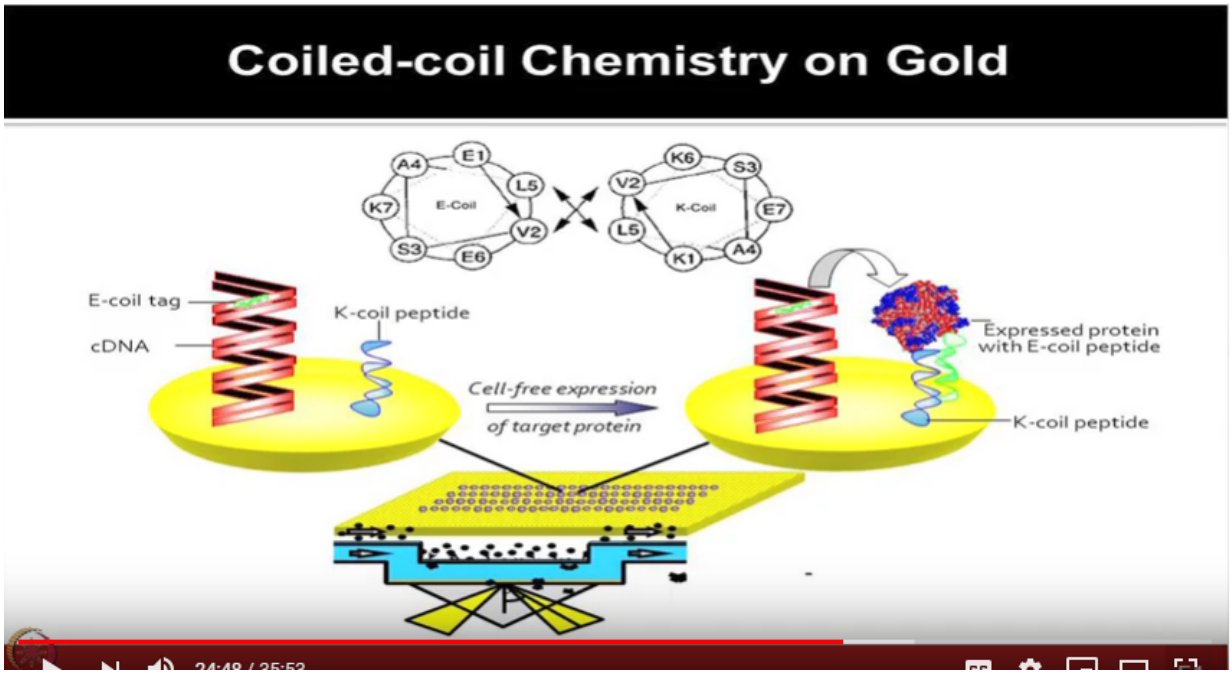

So, then there were no a newer approaches, came which was one of them was quite a welcome chemistry of using, a coil and K coil, can our clones may have, you know e coil and the chip, may have the K coil and using those particular type of chemistry, now if you chip we don't print here all the clones, using the same NAPPA concept, but now you have the K coil a to the captured were paid and each of the clone, can tasty coil tag. So, you are not talking about peptide, peptide interactions here and it's a very no strong interaction, not having so, much material printed on the chip surface. So, you have you don't know 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. I mean what you are 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 he tamer is being formed now you will have the tight interaction, you can see this protein synthesized on the chip surface and which could be, then detected and used for the further interaction studies.

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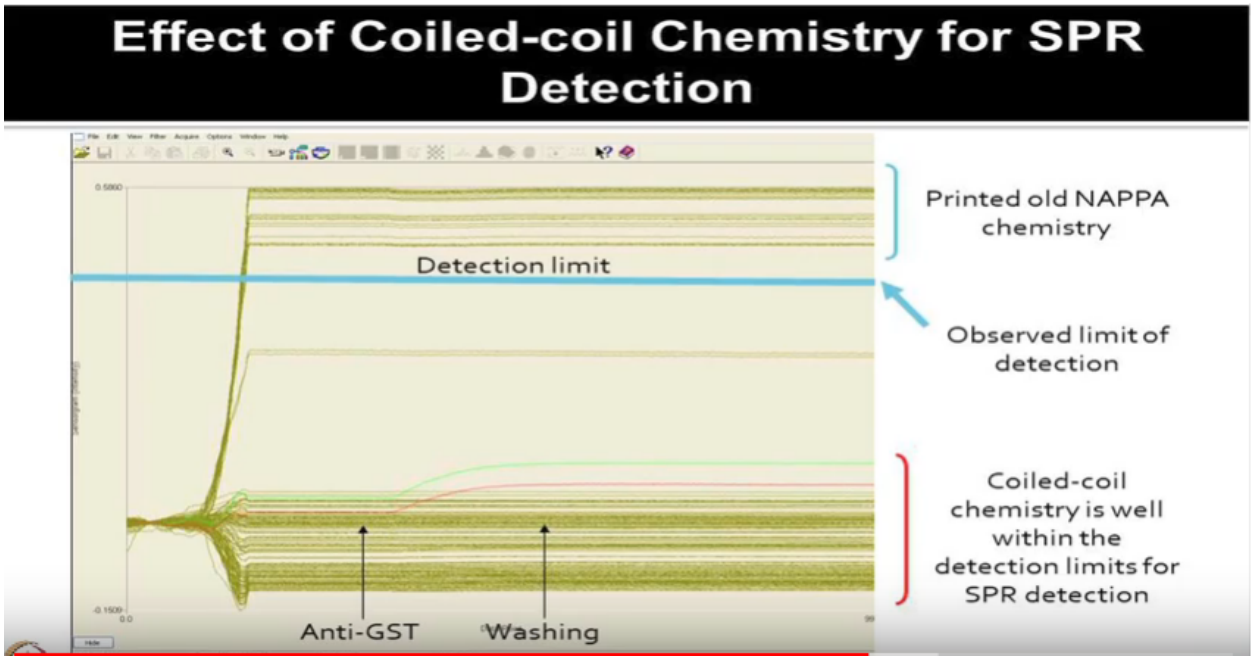

So, now the baseline which was seen here earlier, now this same baseline is much lower here, with the new chemistry. Right? So, the old chemistry, but still the new chemistry and now you can have much more room for measuring, any kind of binding responses, you can change different concentration and you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

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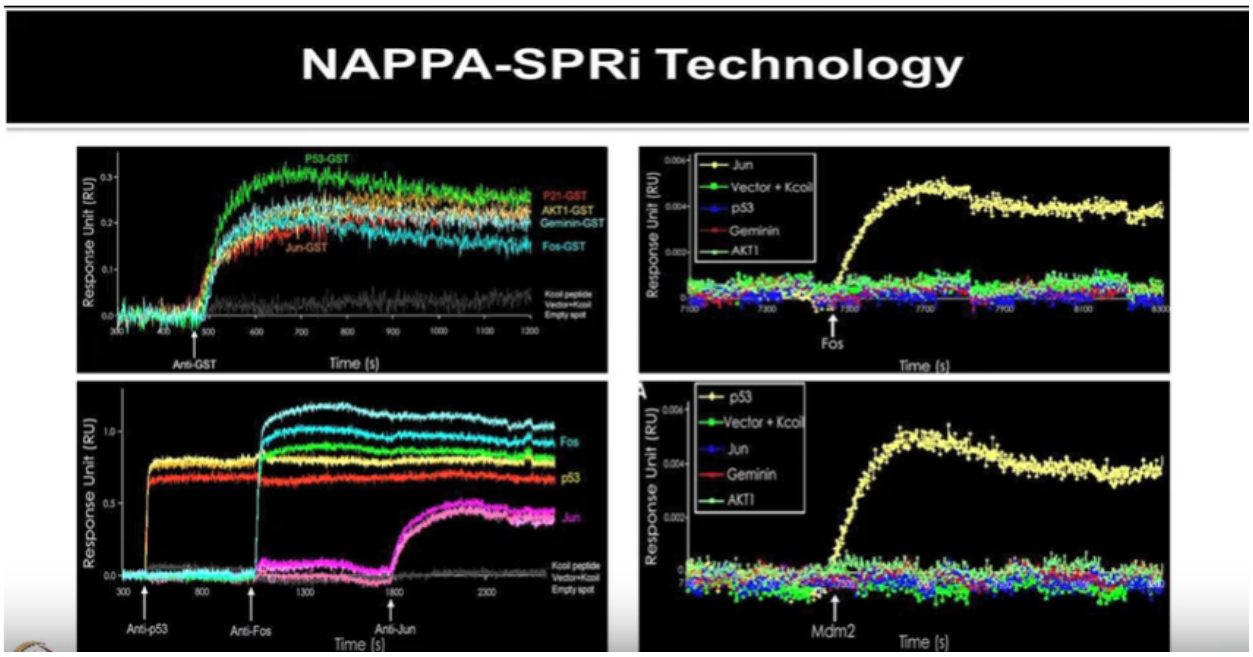

So, several experiments were done, in this case initially you know various type of proteins, were tested for expression first of all, whether on the gold chip, with the new chemistry can we detect the protein expression. So, every protein has the GST tag. So, in this case you know handful of eight or so, protein which we are 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 it's starting from anti p53 and they force antigen antibody on the same chip and now if you can see that replicated spots are showing binding, in response to those particular antibody which you're injecting, then probably this binding is not bulky effect, you are what you're measuring, is protein specific antibodies you're using. So, protein specific responses, you are observing. Right? In case of GST it can it can be something you know everything is showing 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 for SAR Jun then probably you have a specific ,type of you know measurement which you can make here and then you know some protein interactions were tested using, Jun and false as a pair, phosphor the injected and you know as No printed at the cDNA on the chip and there was combining observed, similarly mdm2 and p53 were tested out and then you know those kind of things showed some binding.

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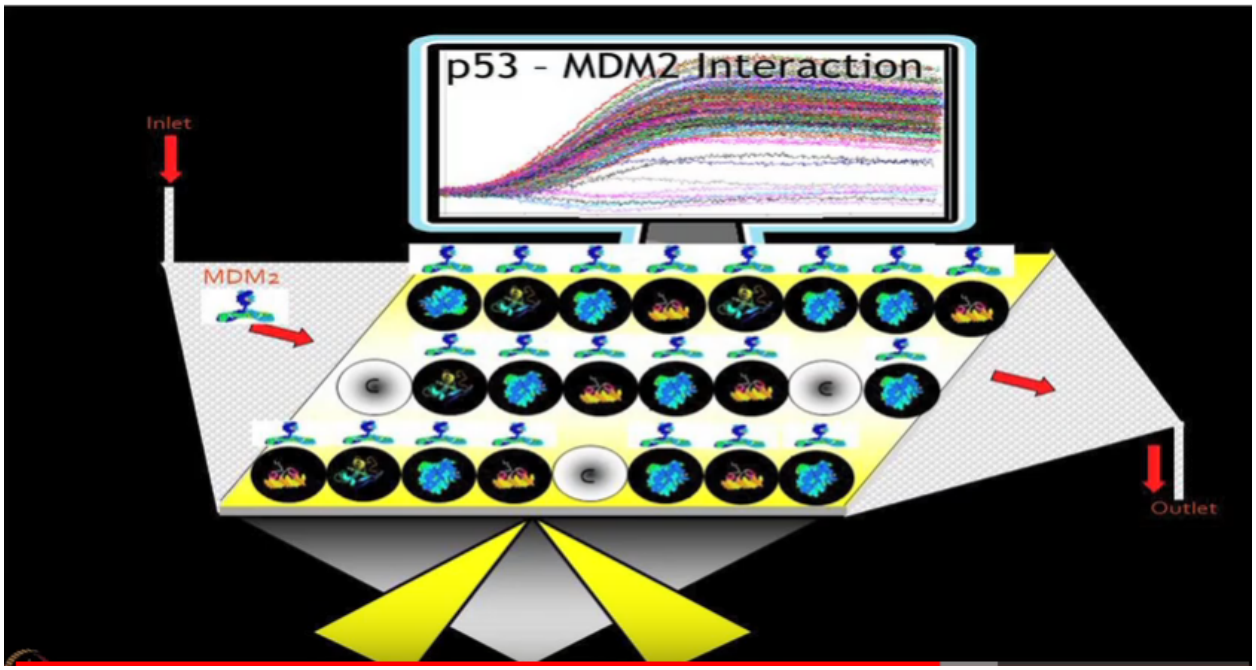

So, you know an ex experiment, is just planned which was more kind of high-throughput experiment and which was very challenging, to really do that thing on the prototypes, is what we had available, where we have you know from one of the companies, in canvas that time gave lot of clones of p53 mutants and these are all hot spot mutants, of you know different p53 regions, to test out into how mdm2 protein, it binds to the wild type of 53, what's a different type of mutant forms of it and our this is a cartoon images to show you, we are trying to look at 144 spots binding, how that can be measured simultaneously,

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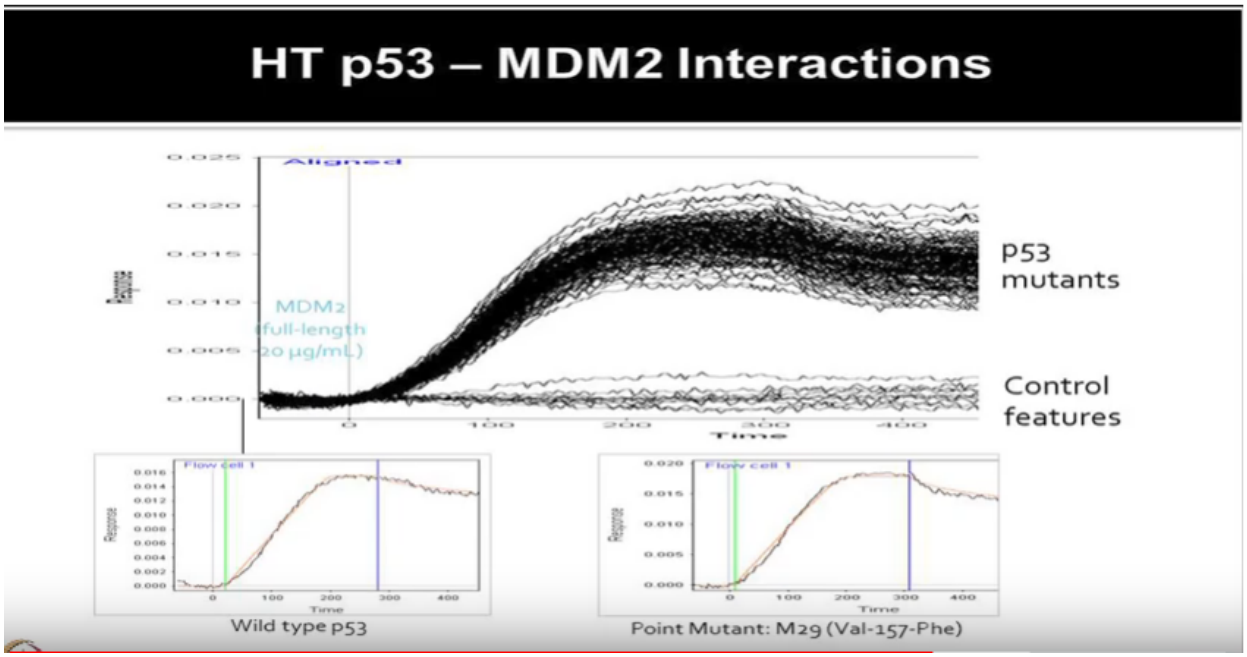

once you do the experiment ,it was very you know first time high-throughput type of experiment being performed, 144 spots binding you are trying to observe simultaneously, but it was very complicated because there was no software available that time, which could do normalization which could process the data. And there were a lot of issues especially we were also finding that you know probably, there was some sort of bulk if it we could observe even in the control spots, there is some sort of a no binding which one could be observed. So, for the next steps of these can technology development, which is even that time we were seeing that even for NAPPA chemistry, there are you know the hello tech chemistry which you are seeing now, is showing much robust sign, also could we now try your the same thing on the you know an SPRi platform, using the device NAPPA chemistry. And that's where now people in the Josh lab are following up the work which they're building a new type of fresh SPRi platforms, where hello tech based chemistry could be using the haloakanes 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'm 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 interact or on a SPR this is what you do you have a known protein which you mobilize, then you are floating, one of the potential known protein to find out interactions. So, then you are not doing much justice, to your actual experiment and the technology, because you are already knowing that things so, much and then you are okay saying, these two are should be binding, but now I'm confident they're actually binding; this is what you do in a SPR. Right? What can we actually of the technologies, that you have an unknown protein printed and now you are passing, your, you know a 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 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 realize, you read enough of the peptides, which is required for detection, of those but those peptides and the SPR and when you are doing these things that you know just very low concentration, that's not sufficient for you to, give you an appetite for doing experiments. So, it's 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 in tractors, 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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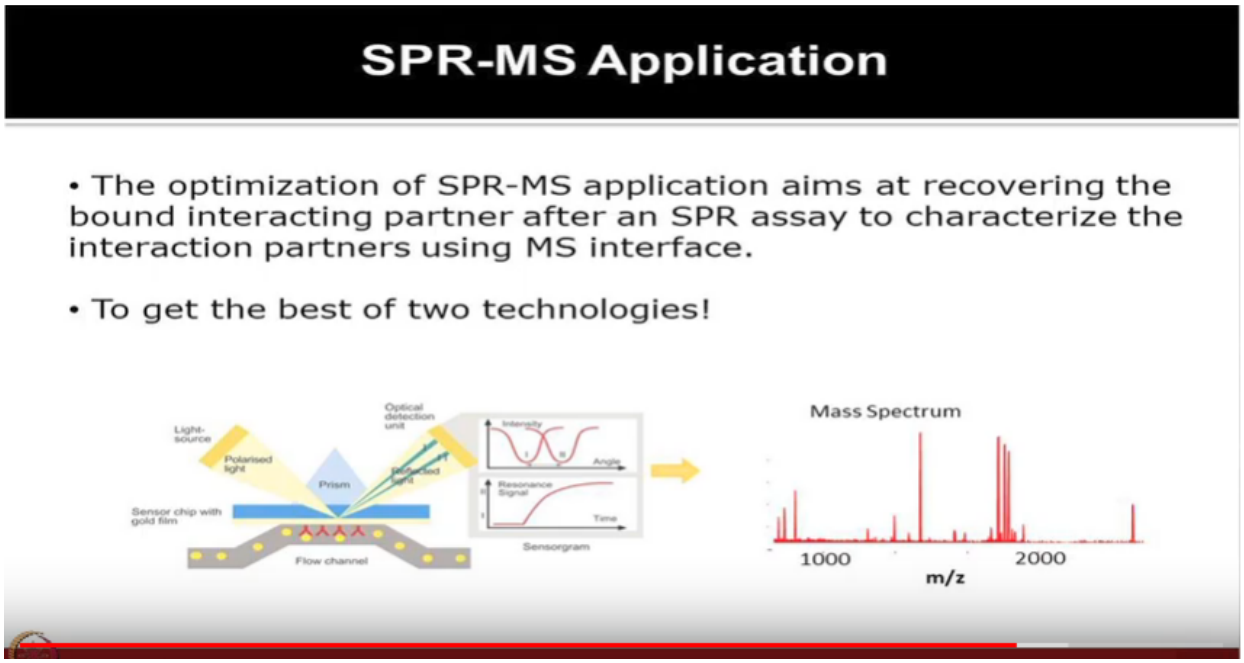

So, the concepts are you know very straightforward, we are employing one of the SPR technology and one of the mass spec, you know technology two together, trying to get the best of the two technologies, to identify they're known in tractors.

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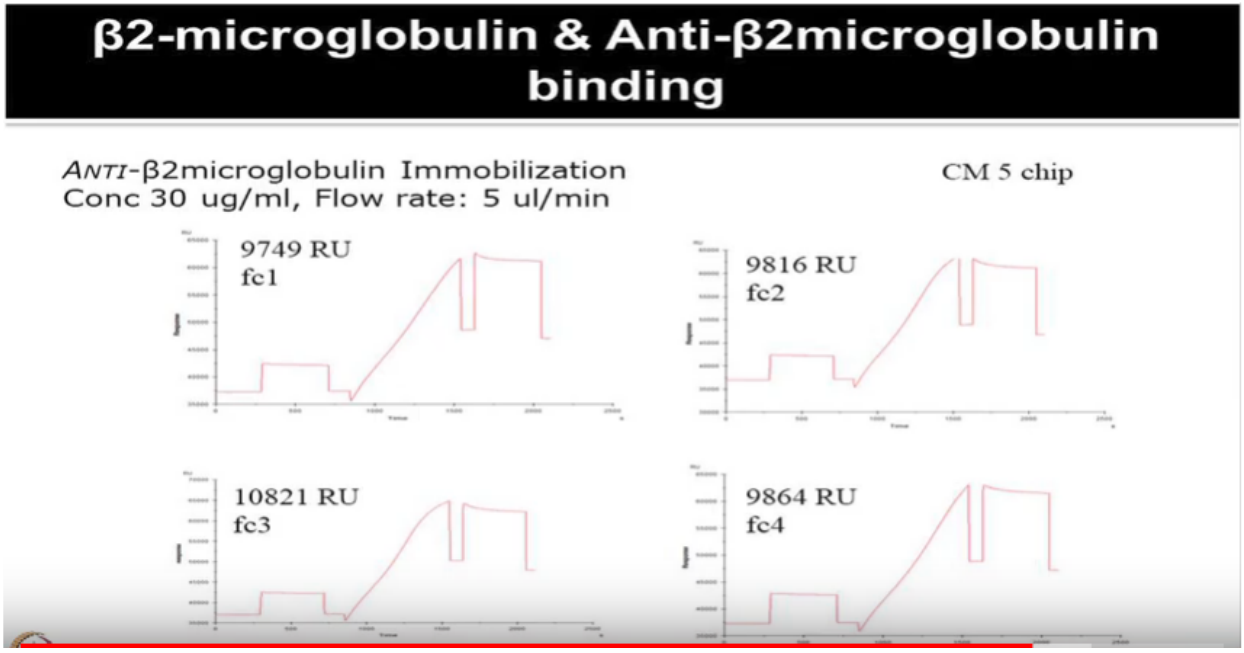

In this case again we started as a proof of concept, which are2 microglobulin experiment and then use all the four flow cell because you need, lot of analytes to buy in which you can recover and then use for the mass spec testing. So, all the for flow cells were immobilized and now when you are passing your B2

metal glob in protein, then you are enriching them and we actually, you know use it pretty high concentration 30micron per ml, flow rate was used 5 micro tree per minute, all the four flow cells were employed.

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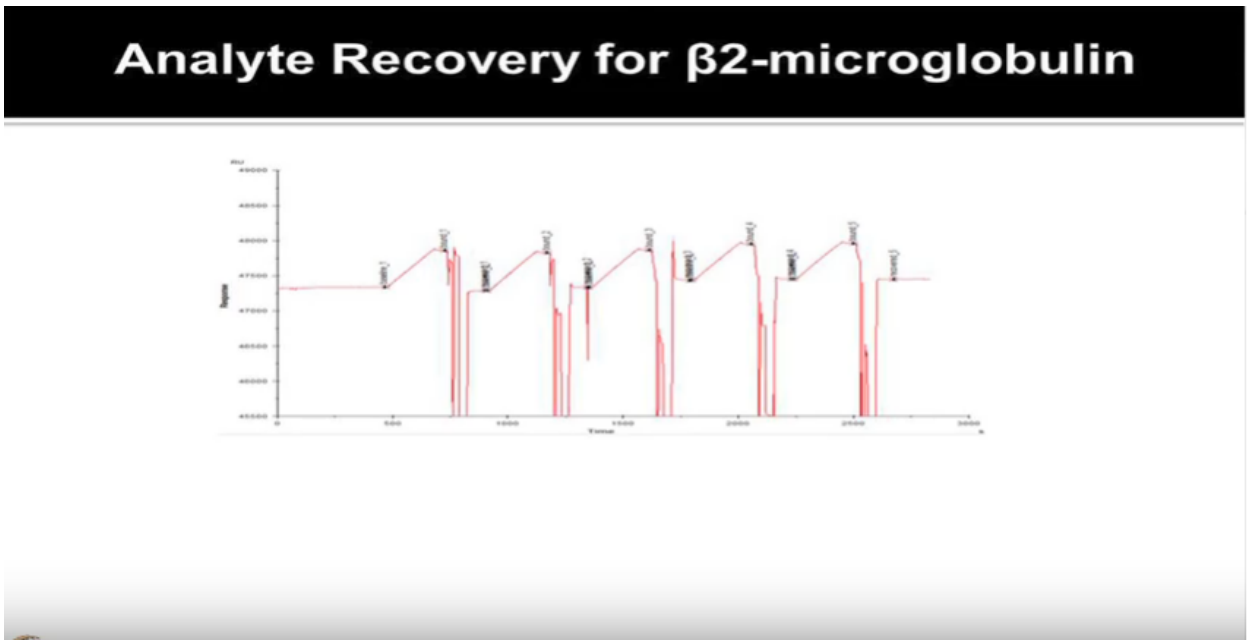

After doing you know series of cycles so, we, we did that for maybe almost 20 hours or so several rounds, on all the four flow, cells with intention, that you know enough of the protein should by in with a microbe 11should bind on the anti B tomato globulin. And then can we chop off that particular bound protein, with a microglobulin then get peptide is settled out of those proteins. And then finally we can detect them using mass spec that was the objective.

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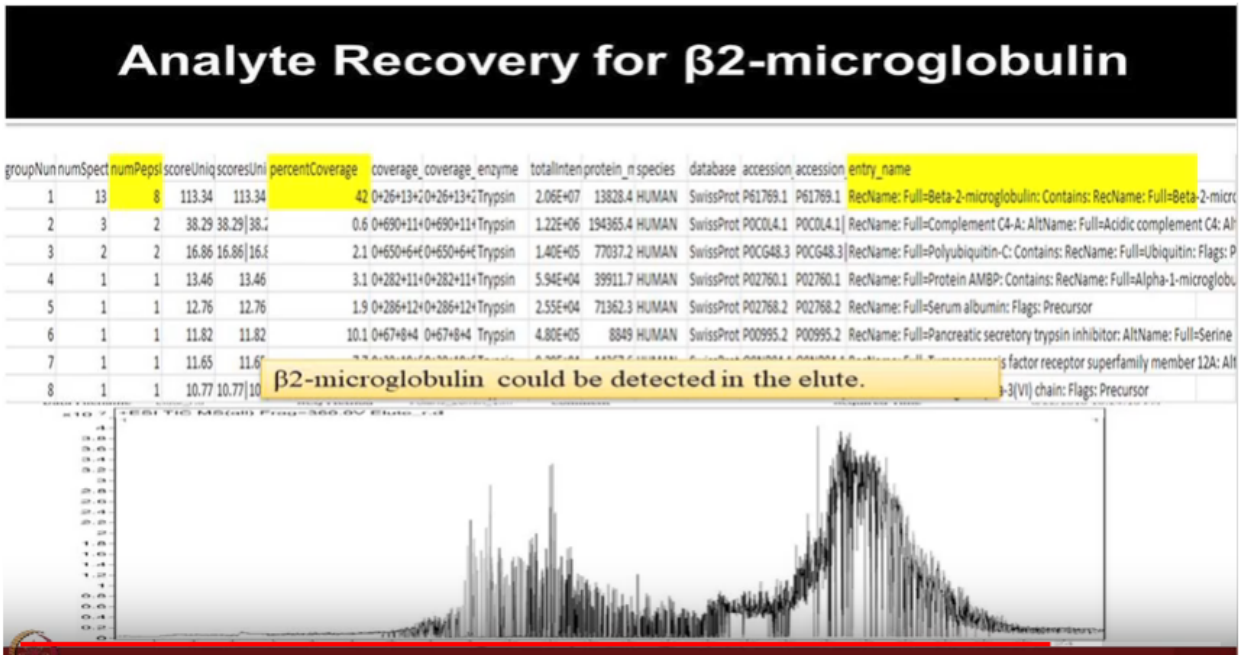

So, after doing mass spec successes, not so, great but it still we could see eight peptides, coming out of the same protein of interest at the top hit, with a meter to micro Logan you can see here, as a first hit of mass spec this is the kind of chromatogram, which we saw.

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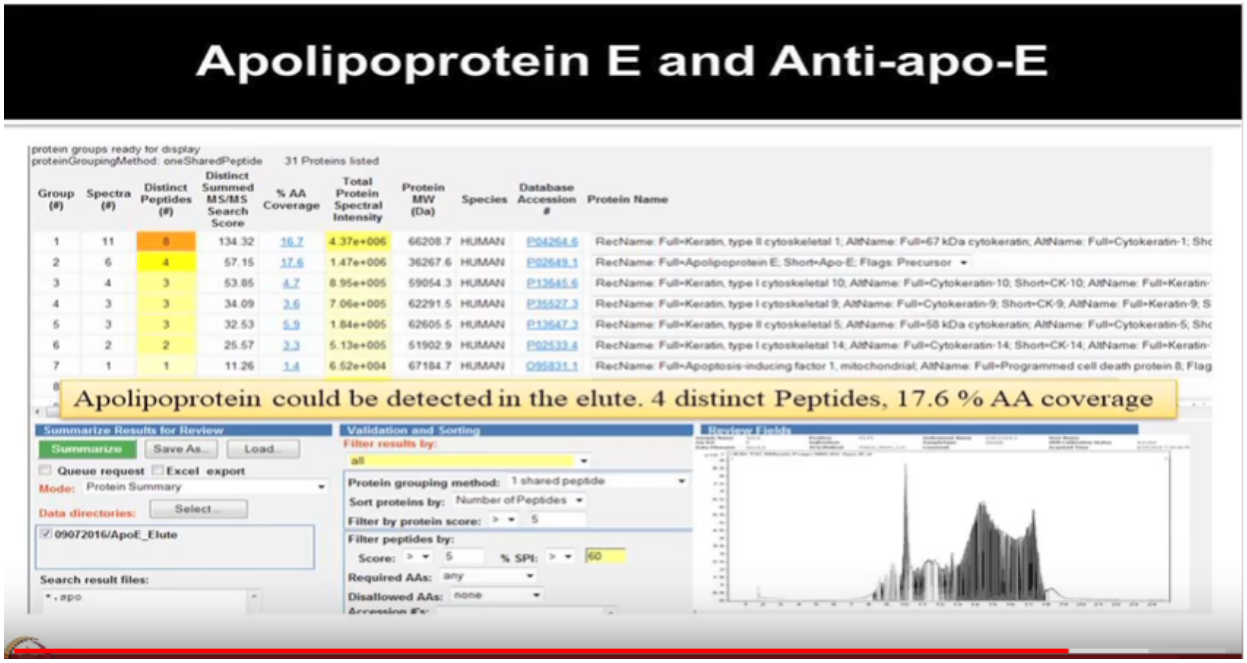

So, then we tried on another protein, which was of the physiological interest apolipoprotein e and intention was again to you know look for their binding, this time you could see four distinct peptides. So, at least you know good news, was and this one we are using cute of platforms, not the latest or biorap technologies. So, we could see the right protein and we detect the right peptide which was good news, but of course many times you do not have opportunity to run, all four floor 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 Bella scale experiment, when you really want to identify the unknown interact it's, we receive binding but you do not know the protein of interest. So, you can employ both SPR MS platform.

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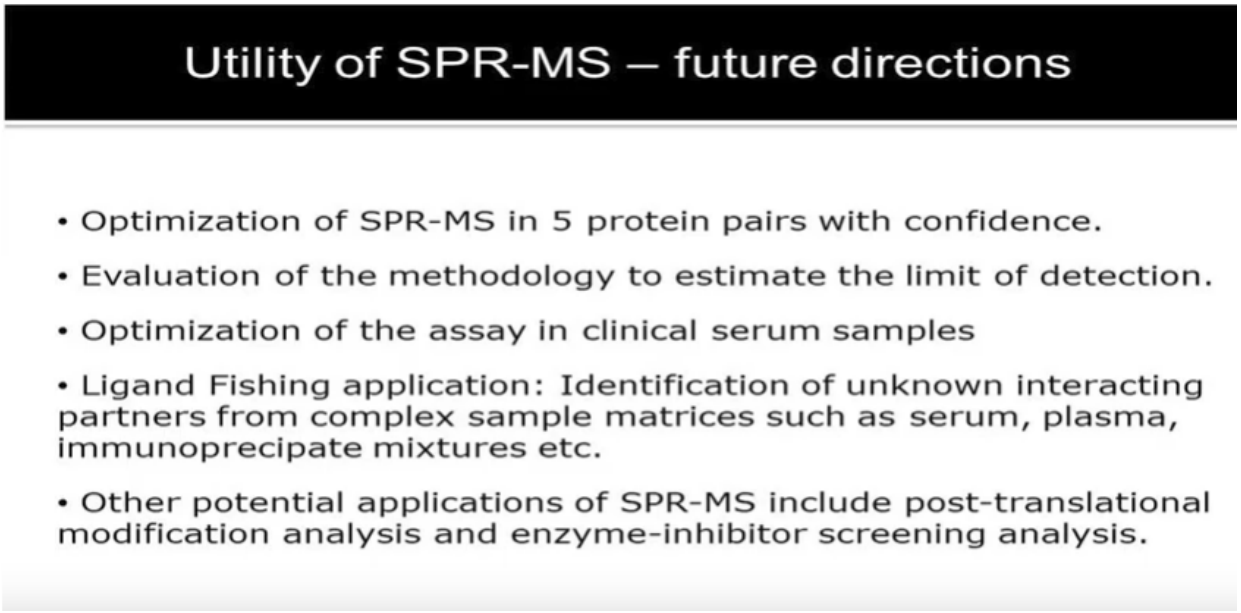

Albrights'? SPR ms you know now we have at least tested as five protein pairs, which looks more confident, we are kind of alerting those technology to see limit of detection, what could be best used and some of these could be you know heavily, useful for the clinical has sale especially serum type of samples, you have a known protein of interest and you want to see you know where it binds, with other integrators. So, a lot of like in fishing applications can be you know, thought of bailed on this application, which is what we are testing out and some of the G application scientists areal. So, working with this so, this particular technology having lot of applications, including it could be used for the PTM kind of analysis, as well as in the inhibited screening analysis, but you know not 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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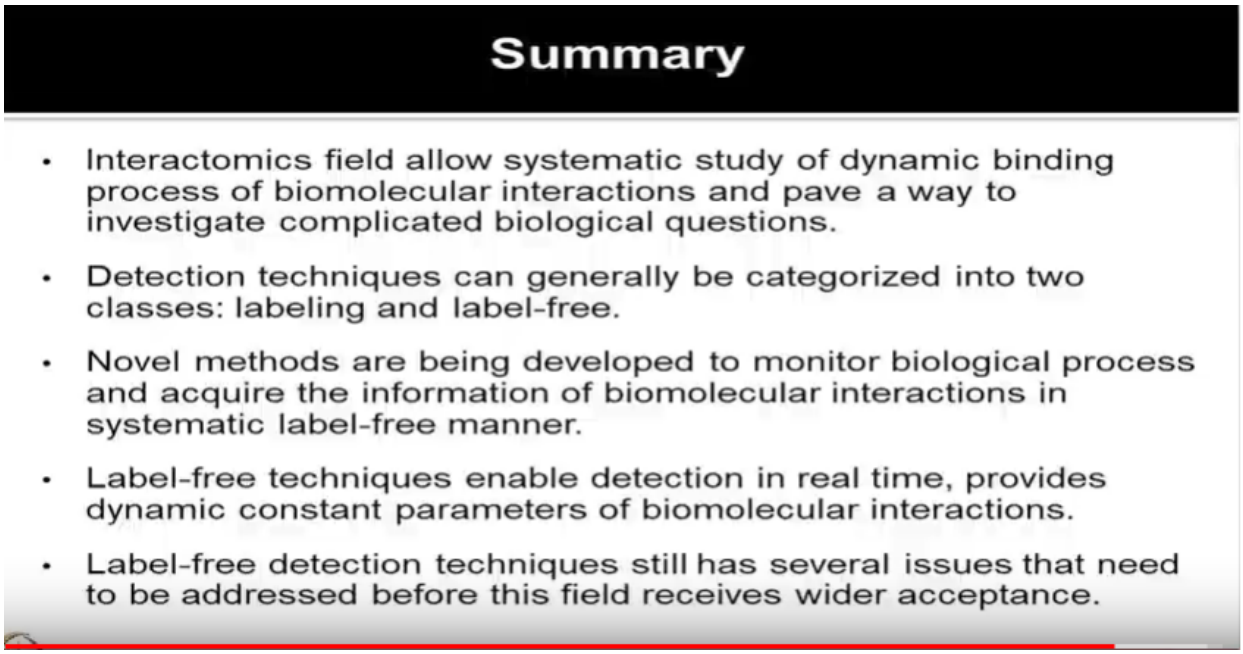

Somebody I think I have given you probably a broad overview of various inter atomic field, you know in which way, the dynamic molecules to really understand, them you need a gamut of technologies, you cannot just rely on one, that I have studied in this course and I can now start going using for every of my application, you have to very open, to look for various type of platforms, which are available to you I'm sure with overall you are probably convinced, that you know there is not a potential, of using these technologies, they built free technologies and the different platforms, available thing from, you know looking at conductance based principal defect and base principals, 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 findings, 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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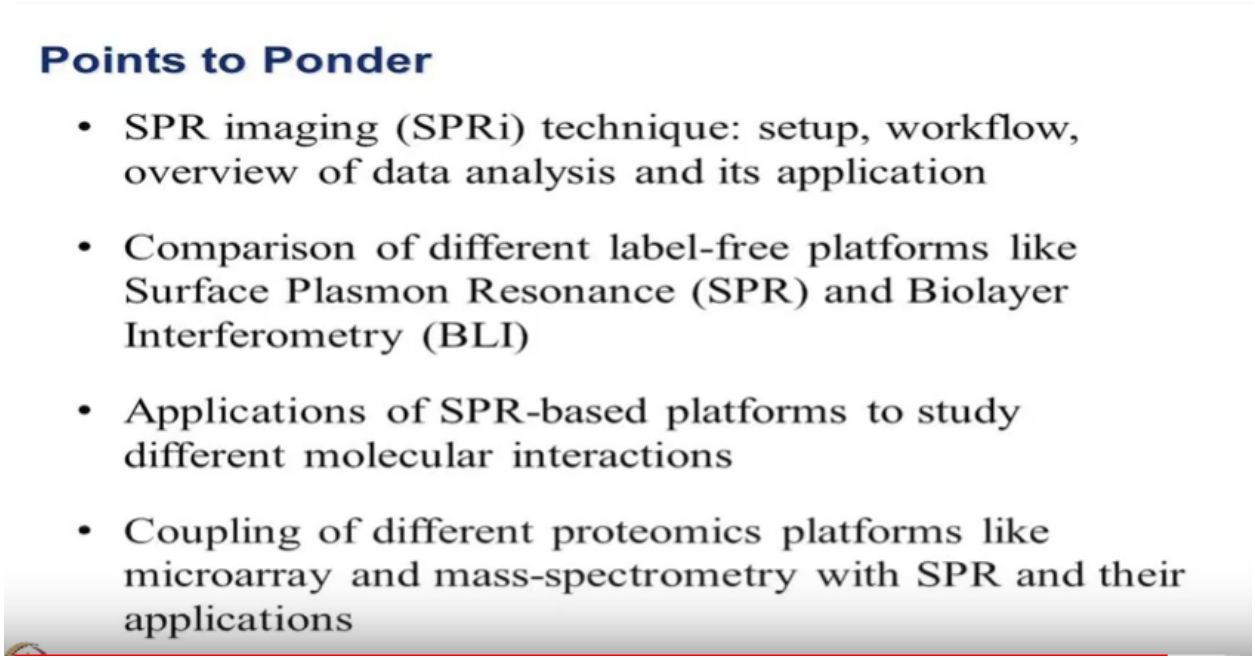

I hope, now you are able to understand and appreciate, the utility of label-free technologies, in the field of interactomics and also integration of novel technology platforms, especially SPR imaging, SPR with mass spectrometers, as well as other technology, platforms in the proteomics areas, which could be utilize 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 interaction or protein drug interactions, in the upcoming lectures, we will also discuss about coupling of immuno precipitation, with mass spectrometry, to detect and identify, the interacting partners and the molecules of your interest. I hope these novel technology platforms, which you are discussing, in the last two lectures and in the upcoming lecture will make you much more aware of, available technology platforms, for your applications. Thank you.