NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING (NPTEL)

CDEEP
ITI BOMBAY

Applications of Interactomics using Genomics and Proteomics technologies

Course Introduction by
Prof. Sanjeeva Srivastava

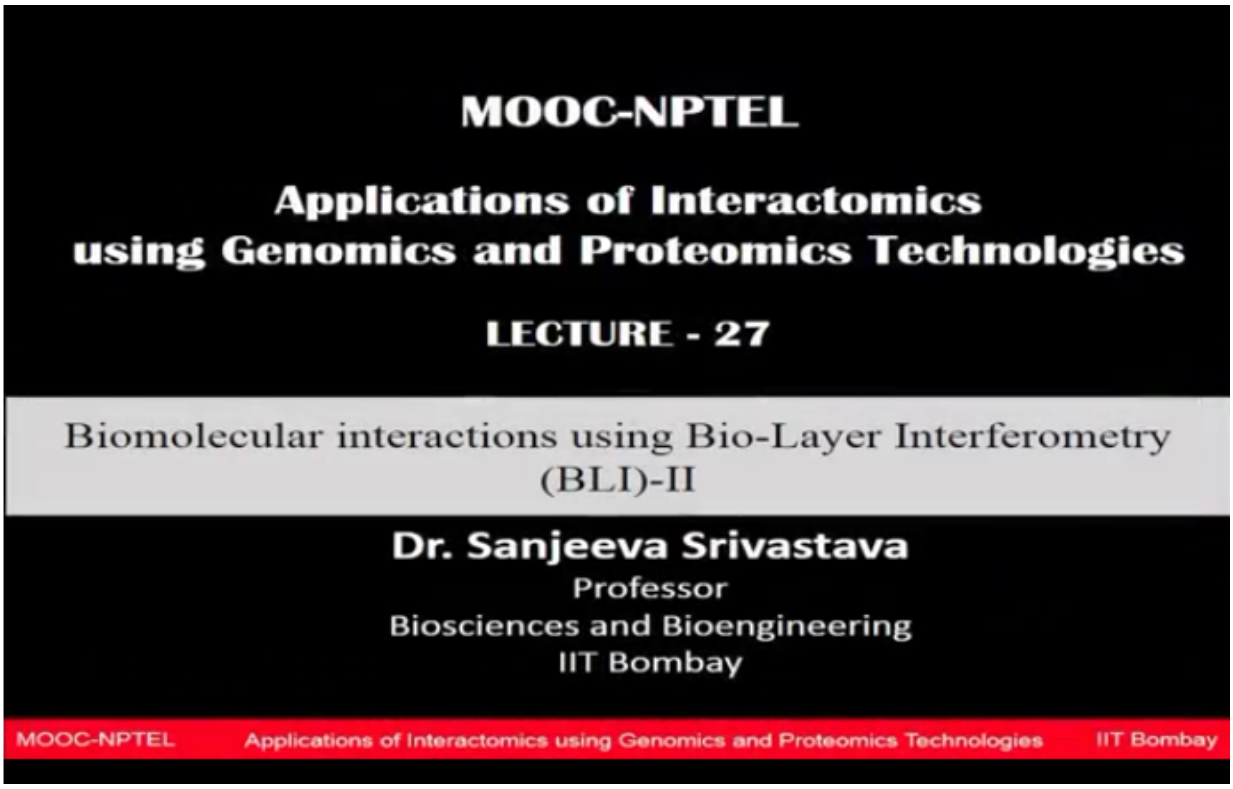
MOOC-NPTEL

Applications of Interactomics using Genomics and Proteomics Technologies

Lecture-27
Biomolecular interactions using Bio-Layer Interferometry (BLI)-II

Dr. Sanjeeva Srivastava
Professor
Biosciences and Bioengineering
IIT Bombay

(Refer Slide Time: 00:27)



Dr. Sanjeeva Srivastava:- Welcome to MOOC course on Applications of Interactomics using Genomics and Proteomics Technologies. Today's lecture will also be delivered by Mr. Susheel Vaidya in continuation of his previous lecture and discussion about BLI technology.

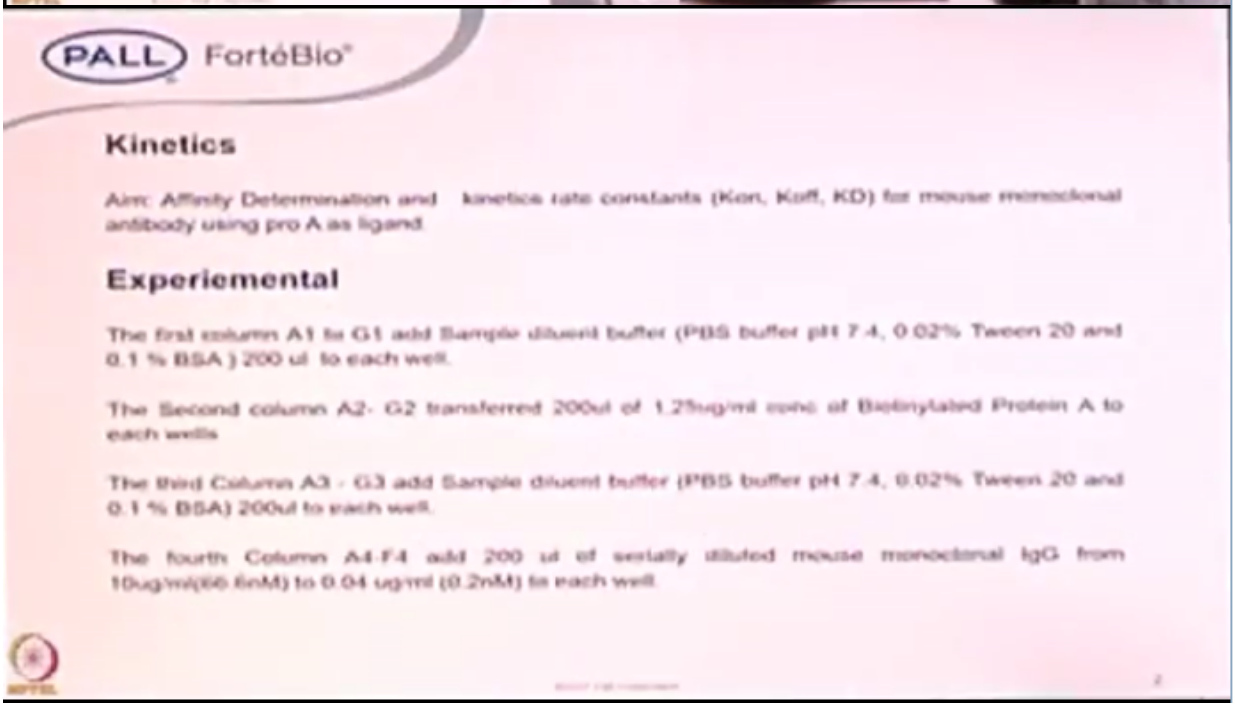
This time he's going to conduct the hands on session and show you how to perform this experiment on biological samples of interest, I'm sure now you will be able to understand the principles much better and also get a fair understanding about the protocols and the technology how to use that for your experiments.

So let me welcome Mr. Vaidya again for this demonstration session which I'm sure going to be very stimulating for you.

(Refer Slide Time: 01:18)

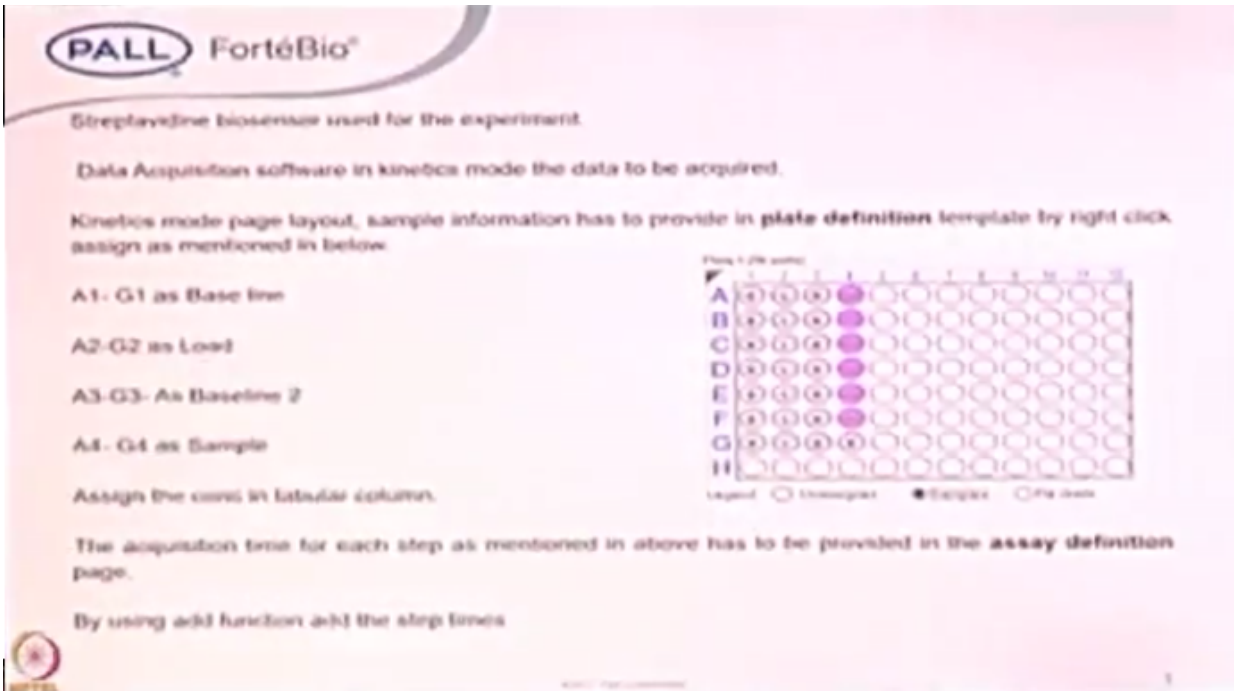
Mr. Susheelendra Vaidya:- They had seen the BLI presentation what the technology behind in the BLI as well as the few applications, now we can do the experiment how easily we can do the kinetic studies using the BLI technology, sorry for actually like I was planning to project the screen on the projector, so but unable to do that, so using this we can, with connected to the server, so work station we can do the experiments.

So what exactly we are doing here is the first experiment I'm going to start is the kinetics here, (Refer Slide Time: 02:04)



that kinetics experiment what I'm going to do is the first experiment, so kinetics and affinity measurements using the first experiment we are doing the affinity measurements as well as the kinetic rate constants for the mouse monoclonal antibodies using the protein A as the ligand, what I'm going to do here is the first I'll put the first column in the 96 well plate here the buffer, the buffer is the buffer the sample radiant,

(Refer Slide Time: 02:35)



and the second column I will put the biotinylated the protein A as a ligand and the third column once again I put a buffer, the buffer is here is the SD buffer the sample diluent buffer which consists of your PBS, PS7.4, as well as the tween 20, 0.02% and the 0.1% BSA.

So why you can ask so many components are in the buffer, so generally why we are adding the surfactants as well as the BSA like, it reduces the non-specific interactions, generally protein have a chassis species, have a tendency to bind to the any solid support, to reduce this nonspecific binding what we are doing is we are adding the surfactants and as well as the blocking agents like the BSA. In case of the ELISA you are adding a BSA or the surfactants like tween 20 or the triton X100 or any other surfactants like NP40 and all that.

Now we are using same thing in the buffer systems, the PBS buffer, it is a PS7.4 as well as consists of the BSA as an tween 20.

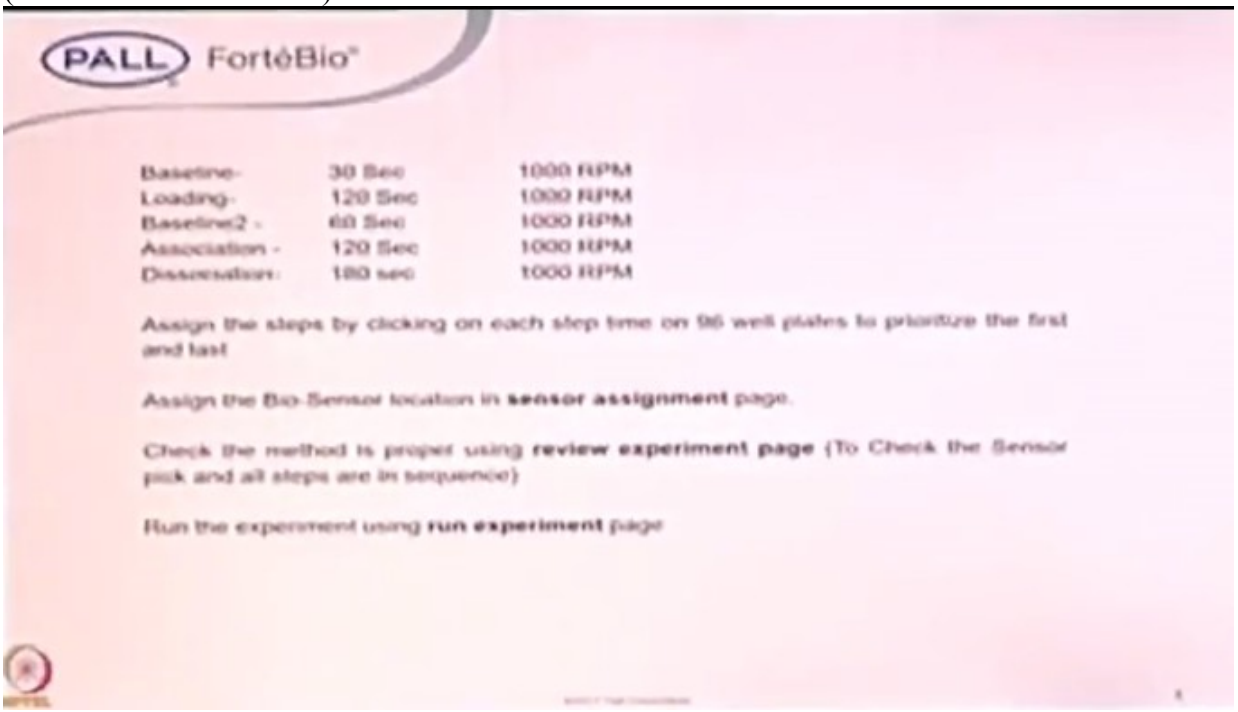
And then the last column if you look at the sample column number 4 is your mouse monoclonal antibody, we have a serial to 3, 4 dilutions we will do it, and then we can study the interaction between the mouse monoclonal antibody to the protein A ligand, so this is what the experimental plan, what the concentrations we can look at before that, the biotinylated protein A ligand I will take it around 1.2 microgram per ML that much concentration is good enough for the immobilization of the biotinylated ligand protein A, and the mouse monoclonal antibody I will take it around 10 microgram per ML is the concentration is equal to around 66.6 nanomolar, using 66, that is the highest concentration we will go 3, 4 dilutions using that, around 6 titrations series we can do using that.

So one last column I'll put it as the blank buffer, just only is a D buffer, so why we need your referencing is like that sometimes even the buffer can gives a artifacts, system can generate artifacts, to substrate those datas we need a blank reference, so we can, with a using a 7 probe we have a 8 channel we can either go with a entire 8 channel or just only 7 channel or you

wanted just test it or a single sample that is also possible, it is need not like that you have to use, if it is 8 channel you have to go with a 8 channel, if you have one sample with a one sensor you can do it or 3 samples you have a 3 sensor you can do it, it depends upon the how you are going to design your template.

And these are the concentration, very simple, and I'm taking about the time around 30 seconds is my baseline time, and the 120 seconds I will look for the loading how much load it happens on that, and again the software have a compatibility like you can extent when you are,

(Refer Slide Time: 05:34)



during the acquisition itself you won't extent some more time, it is possible or you want you see that there is a saturation happens then you want to just jump to the next step, yes that is also possible, this feature is not in case of the SPR, once you fix this time you can't change, but in case of the BLI technologies it's possibility like that we can play around with a timings.

And the next baseline I will do for a 60 seconds where, once the immobilization is happened then the unbound molecule you want to wash off, we need a stable baseline then we can using 60 seconds is good enough, I will do the 120 seconds association where your antibody binds to the immobilized protein A, and then dissociation I will do it around 120 seconds.

The three around to 5 minutes around roughly if you take it around hardly it's not more than 15 minutes, so in a 15 minutes we will finish off your entire kinetics experiment in a one goal, in comparison with SPR so one injection say suppose after immobilization it's a 3 to 4 minutes immobilization, then each concentration say suppose you are injecting a 1 nanomolar solution, 1 nanomolar concentration, 120 seconds is 2 minutes and then the 3 seconds, so totally 5 minutes is the one concentration you have, so if you are doing for 6 concentration it is roughly half an hour time, more than half an hour it is, after the regeneration then washing then injecting the same samples over the say immobilized surface it is roughly more than a 1 hour

experiment, but you can quickly finish off in a 15 to 20 minutes of time, so where you can get the fast, the determining your, the kinetic rates constants and then easily replaced screen the experiment which is the binders which are not binders in case of the screening experiments, if you are doing the full maturity kind of experiments where you want to get the full kinetics pattern then you can quickly develop a method and then get the KD value.

In comparison I can say that why the SPR and the BLI's are faster, say suppose when you are developing a method you don't know how much I have to immobilize on the my sensor surface that you can quickly screen on the sensor, on the different concentration if I put the immobilizing molecule on the sensor surface, say suppose 1 microgram per ML, 5 microgram per ML, 10 microgram per ML, then you go for the further association, say suppose 1 microgram per ML I'm getting a response of around 0.5 nanometer, and 5 nanogram, sorry 5 microgram per ML concentration also I'm getting it around 0.5 nano response, then you can compare, so if I whether I need to immobilize higher concentration or lower concentration, because 1 microgram per ML also getting a 0.5 response and 5 microgram per ML concentration is also getting a the 0.5 response, then the question is whether you required to overload on the sensor surface using the concentration, so what happens is in the label-free platforms if you are more concentration you start immobilizing on the sensor surface there is a possibility that steric hindrance happens, there is a one molecule binds here, there is a one more molecules immobilized here, and one more molecule, so your binding pockets are something like very closed to each other, there may be possibilities that due to the more crowding on the sensor surface there is a possibility of less binding or sometimes it leads to a nonspecific binding, or you can see the biphasic associations, these are the different ways when you are looking at the interpretation of the sensorgrams what we call it as we are getting the data, the binding curves, we call it as sensor grams.

Looking at the sensorgrams whether it a 1:1 binding model or a 2:1 binding model or 1:2 binding model, so easily we can look at it using that sensorgrams, so now we can start the experiment, it's just only the sample preparation time takes some in that, but the operation time on the instrument is something like very quickly you can get the data, so is anybody would like to do with me for the sample preparation? Fine, yes please.

So this is the 96 well plate black plates we are using, it is not a transparent plate, it's just a plastic plate, polypropylene flat bottom plates, we are using in the entire experiment, why you can ask? Why we have to use a black plate rather than the transparent plate, the question is here is it is works on the interference based technology light will be pass through the sensor so I can show you these are the sensors, typically is like this, if you can come around here you can see that how exactly it looks like, or later also once the experiment is done even you can look it into that, these are the typically your sensors, either you can pass this tray to everyone there how it looks.

So typically its looks like your injection needle, everything is happening at the tip of the surface, the interactions, just the surface area of this the fiber optic is just only 600 micrometer and diameter, okay, well capacity is 390 and above, sorry 395 divisions, but the minimum point what we are looking is to, so even we have a _11:24_ okay the sample, so it consist of previous BSA 7.4 and tween 20 as well as BSA in that, so adequate 200 microliter - on side by side what

we were doing is here these sensors are actually a dry-coated the streptavidin, what I'm using this sensor here is to streptavidin, okay, so I have bio-molecular protein, so what I'm doing is these sensors I will hydrate with this number, so the minimum hydration time is a 10 minutes.

So ultimately 200 microliter is, yeah, the first problem 1 and problem 3 depend on 200 microliter, 200 microliter each problem 1 and 1 to 7, so on side by side what I'm doing is I have taken a one more plate here, I had already added 200 microliter each of the same profile, I'm placing this while you can see, so this is the sensor tray, this is the 96 well plate format once again, so these are the 7 streptavidin sensors I had taken here and then I have kept and I have put it like this, so it will make that, okay, then you add problem 3, it's only the time is that making the plate, that's all, the rest we can program it is un-attendant just to leave in that.

Unidentified Speaker: You didn't add anything?

Mr. Susheelendra Vaidya:- No, no, I added just 8 per ML, we are doing with the 7 sensors, okay, then now I have a protein A here, the biotinylated protein A it is 250 microgram per ML, okay, same way around 15 microliter from this, then make up to 1500 microliter.

Unidentified Speaker: We should let others to do it.

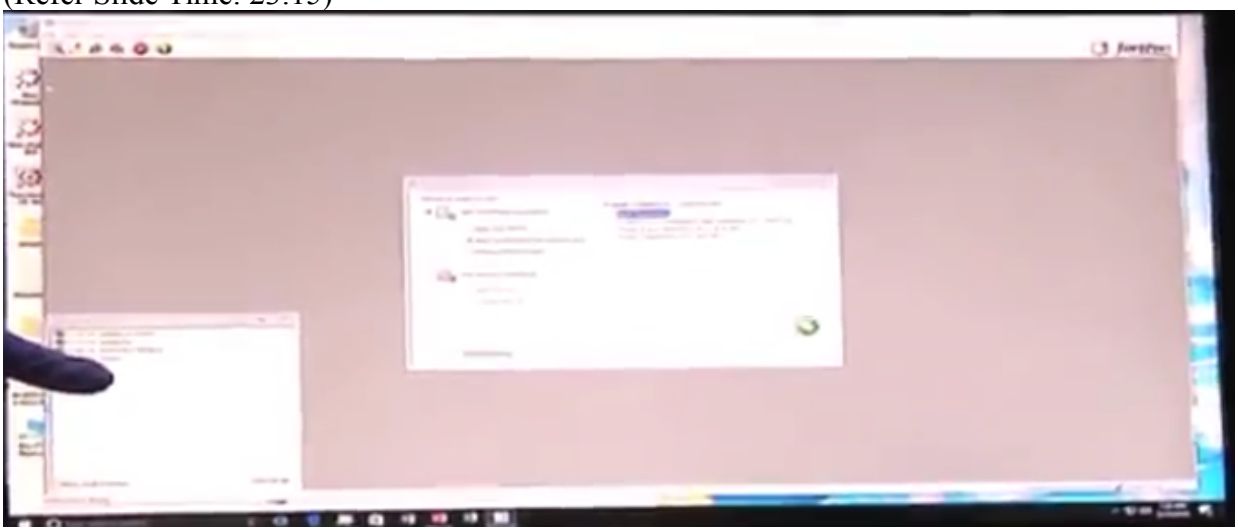
Mr. Susheelendra Vaidya:- Yeah, take around, the 15 microliter diluted to 1.5, and I have a mouse monoclonal antibodies, it is 1, I'm having, okay I have 20 microliter here, okay, 10 microliter, it's corresponds to 10 microgram, because 10 microgram is used into the 1 **_14:40_,** it's nothing but, means 1485 microliter, 1500, corresponds to a, it's a 1.2 microgram per ML of the biotinylated protein, _15:08_, so why I wanted to make you this, it's simple to operate, so then you adequate the 200 microliter of this engagement.

So don't use like a slant, so there should not be any problem, right, fine, done, so now we have to make the binding partner, so mouse monoclonal antibodies, so this is exactly one microliter, I have 20 microliter here, you take 10 microliter or a 5 microliter transfer to the corresponding one angle or 5 microliter, so then I had been taken 5 microliter, put it into 500 microliter, so it's corresponds to 10 microgram and the concentration was 10, so transfer 300 here, and rest when you wanted buffer, and then see, find out what buffer you are taking, transfer and the loading itself, then take 100 then mix it.

Okay, otherwise I'll show one more problem here, okay, so last thing is buffer, transfer 300 here, 200, you can ask that once again I'll transfer here only in this way, first way itself, I can then, I will, I'll take this 100 then transfer, okay, so last tray I kept it blank because zero concentrations, okay, if we had done the plate preparation now we can move on the next, so now the experimental plan we have a two softwares here, the softwares one for the acquisition and the other for the data analysis, so in the first acquisition we are acquiring the data, once the acquired data then we can go for then data analysis, so this two software, so first what we will do is we will go for the acquisition, I'm going to do double click on this, so when you do double click the instruments starts initialization, it's go backend forth so you can see the sound it will make roughly around 30 seconds for the initialization, in comparison to SPR when you start the experiment you have to like pass the buffers, stabilize the flow, and all that, here

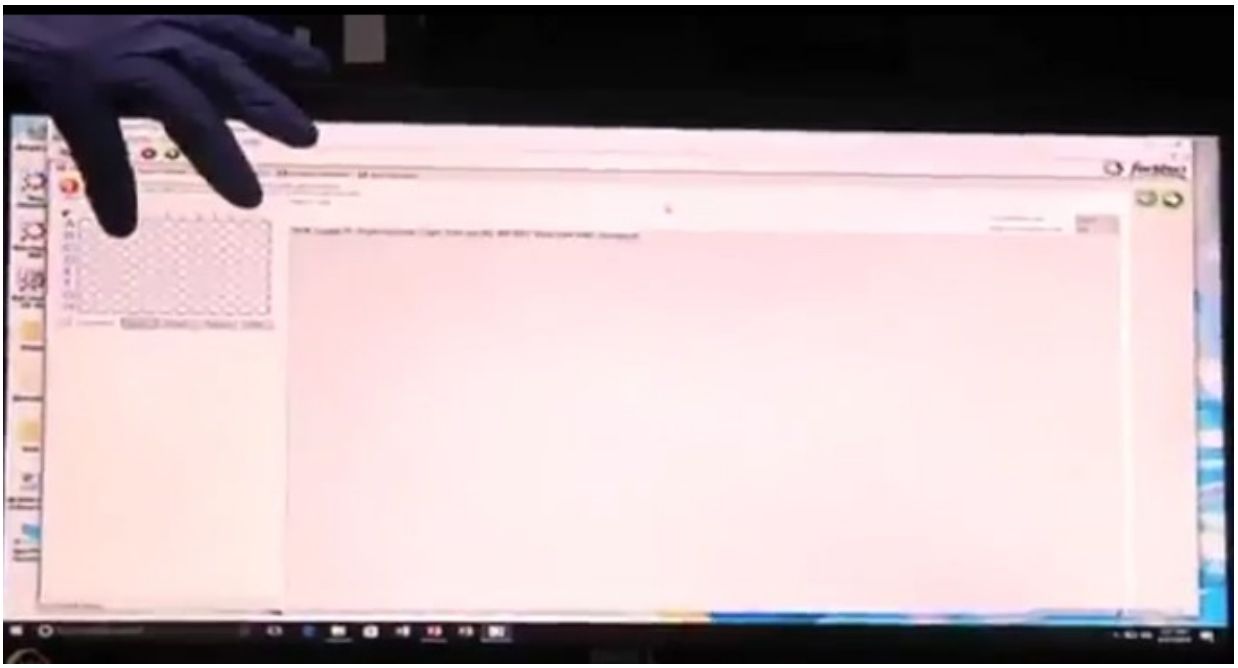
nothing is there just come morning switch on the instrument, allow for 40 minutes then stabilize, just as a lack has to be warm up here, nothing else there, you can now take the data which is showing that initialization.

So if you go X, Y, Z direction alignment then it will comes a message as a ready here, this is roughly 30 seconds initialization, so now you can see that the instrument is ready,
(Refer Slide Time: 23:15)



so we can start the experiment.

So when you click on software that which data, then you can see there is wizard, in the wizard what kind of experiments you wanted to do, whether you wanted to do a kinetic study or you wanted to do quantitation study, so we have a people the antics as well as the quantitation, so intensities either kinetics experiments, so we can do the _23:41_ software here called that, so as you know we should check the data analysis experiments, then this green mark are at the same group, this is like this I will take a blank experiment here, there are some inbuilt definite are there, but this is the best way to go for blank experiment where you can make your own template model, so if you know the blank templates how to make it your experimental design so there you are going, so I will say you do on a clinger, now you can look like at those the window,
(Refer Slide Time: 24:18)



you look at this is your plate format here, we can see the different frames here, once you call it as a blade deformation, assay deformations, sensors assignment, review experiment and experiments, so these off different, these are the profile actually pages are there you can get designed those, so what exactly the plate definition? You had to feed the information, what are the things you added in the plate, so what I had it in the column number 1, this is 96 well plate pattern column number 1, first we had raised a buffer, the second column we had blue, the third column is a once again the buffer, the fourth column is you had the sample here, the underline, so just we had to assign those informations.

So how we are doing this? Buffer, the fourth is the sample, so assay has the sample, but I'm using only a 7 sensors, not 8, all the 8 sensors, what I will do? I will just select it and remove it, so just I'm using 7 sensors I'm not actually the 6 cause deformations one has to show the, although assign in this last column as a blank a buffer, that also assigned like this, so these are the plate definition, we had to feed here in the template, so in the sample ID what you had feed the buffer, okay, so control C typically it looks like a excel sheet, okay, then the load you have a bio protein A, then the once again you had buffer right control C and then you can go with the buffer then you have a mouse IgG, okay, so here the important is mouse IgG you had to mention your concentration because if you are not feed the concentration you will not get the KD value, so concentration is the one of the parameter when you are calculating the KD, kinetic rate constants, so one of the concentration parameters important, so what is that general IgG KD's 150 kilodalton, so we had to feed the 150 kilodalton here, the molecular weight KD cut off then control C once again you required all the parameter, so my starting concentration is the top concentration is 10 microgram per ML, so just if I type it 10 microgram per ML here the concentration 10 microgram per ML software have a, you can change the concentration parameter like concentration, microgram per ML or a MG per ML or anything, and the next even automatically when you put the 10 microgram per ML then when you put the KD value the size of the protein then automatically it calculates the 66.67 nanomolar of your 10 microgram of IgG corresponds to the 66 nanomolar roughly.

So what I'll do is just I'll select all this, okay, I'll rightly click I'll set well data, then I have a option here whether I can go with a concentration basis or the molar concentration basis, a concentration basis is around 10 microgram per ML is the stock, divide by I had done a 3/4 dilution, so what I'll do is just I'll type it 3, 10 divided by 3 then I will choose I had done a programming in such a way that top to bottom, right, then what I'll do divide 10, I'll choose the template here is the down, the black dot is the one which is the highest top concentration, the lighter one is the lowest, I'm using this format if you have feeded this information you can put it in that way or you can low to top then that also possible, then I will say that okay, then automatically when you choose this then you have a 3/4 dilution, so the KD range covering from 66.67 nanomolar it all the way 6 concentration 3 fold it is 0.2 nanomolar, around its roughly equivalent to 200 picomolar.

So these are the things you had feeded in the plate definition, then the next is the assay definition, the plate we had feed, but we have to tell the software you have to go this is the first step, this is the second step you had to assign that, then only instrument move according to the respective of wells.

So what is your, what are all the information we have to feed in this? Here there is a section called as a add, in the add when you go click on this, what we are doing? We are doing a baseline, we are doing a loading, we are doing a association as well as the, we are going to do dissociation, so those informations we have to add, so what is association I had to click it, dissociation I have to click it, baseline by default there is a one baseline is there already, you need a one more baseline is we have 2 buffer columns that's why I'm adding a one more baseline, and what apart from that we have a loading, loading is nothing but the immobilization, so I'll just click it then say okay, so it will ask the step name baseline already exists, because it's already is there, do you need one more? Yes, so these are the steps.

We have to assign instrument go according to the back in forth the robotic arm, so what is the first step here? I will take this arrow mark using this and I'll double click on this, it is just cross marked, so first step is your baseline, then the next step is what? It is a loading, so I'll this arrow mark then once again double click on this it is a load.

Then the third I have a one more baseline too, I'll double click on this, and the fourth is your association, double click on this and the dissociation you can ask where exactly the dissociation happens, I'll do it in the column number 3 once again same buffer I will do it, so 1, 2, 3, 4, 3 the step is, first column the robotic arm move to first, it will dip into the first well, goes to the second then the third, fourth and back to the third, so then you can assign how much time I had to do it, 60 seconds generally I'll go, or 30 seconds or 60 seconds I'll go for the baseline, so just will go for a baseline this one, association time generally I will go for around 120 seconds is just 2 minutes time, and the dissociation I will go for around 3 minutes, 180 seconds, and the baseline to how much you required 60 seconds one more and the loading time how much I'll do it around 120 seconds, let's take it assume just we will take it 5 minutes.

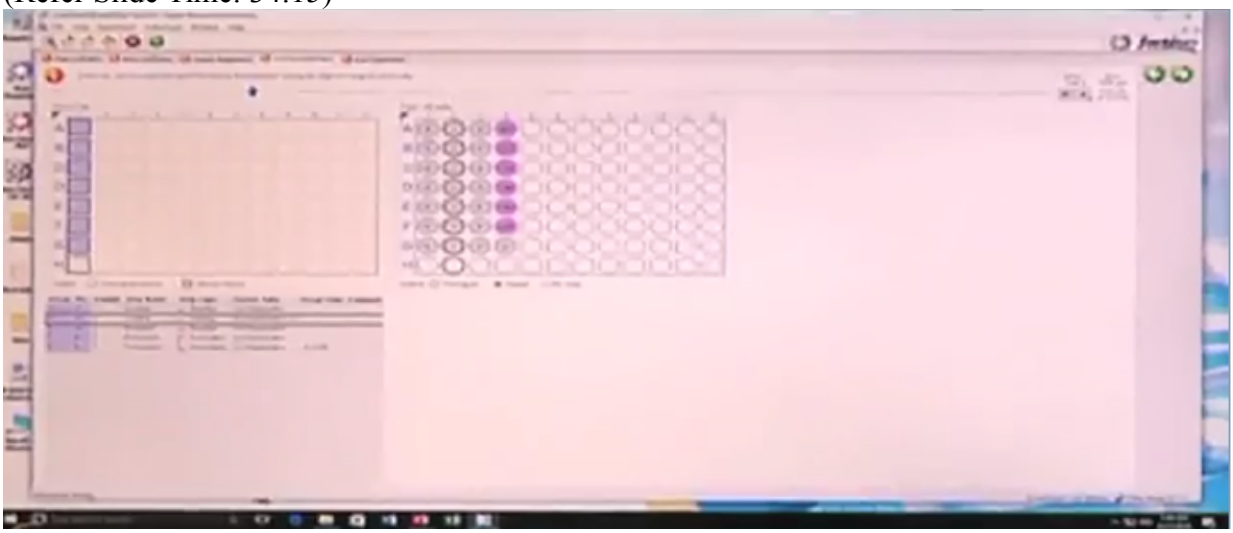
I will tell you these is pure kind of in this one, it has to be based on the experience you had to put it, it's not like the general assumption is like that minimum is 300 seconds, 200 seconds we put it, so just I have put 300 seconds, but in beginning I have mentioned that software have a

option I can jump to next step, if I feel that the concentration is good enough, this is good enough for my binding I can jump to next step or still if you feel that yeah it is not enough, you can extent the time that particular time, still the loading, so I'll put a general around 300 seconds if you feel that it is around roughly 0.5 to 1 nanometer if it is loading is happening, then we can jump to the next step.

Okay, I'm using the streptavidin sensor around roughly experimental time is totally 13 minutes, but even less than the title preferably, okay, these are the according to that sensor will move, and the next is sensor assignment, in my talk you had seen that there is a sensor compartment as well as the sample compartment, sensor compartment where you are placing your sensor tray, the sample compartment where exactly you're placing your sample plates, so you can see here, so this is also 96 well plate format, so I had tell the instrument from where, which column the sensor has to be pick up, okay, by default it is a 1, suppose you had fixed put a sensors somewhere in the column number 8, what you can do is just say remove it and automatically it will think that sensor you had placed it column number 8 it will pick up the sensor from that particular position, and then read, okay.

So now by default I had placed, okay, then when you are doing a QC kind of your experiments and all that you can provide your lot number for your sensors, because it's the very important when you do it in the industry kind of setups where exactly QC monitors those lot numbers what you had tested and all that, so that is the one important or if you have any other information you want to feed that also fine, then the review experiment, before going to start your experiment whether can I, whatever I had setup the experiment is fine or not, so this is your sensor compartment, sensor tray where we have placed this is your sample plate, if you just look it here using this arrow mark just, these sensors are highlighted by the border black colour if you look at here, the sensor is picking from the column number 1 and it is highlighted by the black colour the first well it is going there, then it is going to the second column, then the third, then the fourth and back here, so my experiment setup is fine.

(Refer Slide Time: 34:15)



Sometimes what happens we are running a so many sample, there is a possibility that you will miss the steps, so it is best way to review the experiment, whether my sensors moving in a

proper way or not, if it is something you miss while the number you can go back and then reassign it.

Unidentified Speaker: So that's we have to _34:33_

Mr. Susheelendra Vaidya:- Yes, yes, yes it's a program just…

Unidentified Speaker: And it is just very quickly that's always you know…

Mr. Susheelendra Vaidya:- Yeah, based on the timing here what, this is the review, we prior to the experiments we are reviewing whether my experiment setup is fine or not, so these are the timings I had provided here, so when we are reviewing the experiment before starting the experiment, yeah, and the run experiment the last here where you want to save your data, the path we had to provide, so I'll click here and the desktop I will create a new folder IIT demo, sorry, okay, I will choose this folder.

Temperature we are 23 degree, we are working at room temperature now, this instrument you can work from 15 degree to 40 degree. You can set and allows for stabilization of that, it have peltier, so the next, what is your experiment name? So I will just mention as a kinetics, okay, then here if you look at come here run settings, delay experiment start and the shake sample plate while waiting, what exactly this is? I've mentioned in the earlier sensor comes with a dry actually, these are dry sucrose coating, but we have to hydrate in your buffer, what buffer you are using for your experiment? The sensor has to be hydrate, minimum hydration time is that 10 minutes, okay.

So what I did was during my sample preparation itself first I started a hydration, I started the sample preparation, so I saved my time, otherwise if you forgot the, forget this one, the hydration then you check in this, so after 10 minutes experiment we will start or otherwise if you want another 20 minutes also you can assign the time how much you required.

During the hydration time whether the sample placed has to be in the shaking condition, I mentioned it is not a fluidics based, it works on the dip and rate, so samples will be in the shaking condition, generally not what happens is during the waiting period you are in the shaking, protein have a tendency to aggregate when it's 1, there is a possibility, generally we not advice that, okay, then I'll uncheck this.

During only experiments we required a shaking, during hydration not required, so then you can mention this plate temperature, if you want to work from 15 day to 45 day, now it is around room temperature 23 degree, so even after the experiment do you want to hold that temperature once again back to 23? Yes you can click it not a problem, so after this this is what we have done the experiment, does the setup you can say, then we can say start go, so before going to that we had to place the sensor as well as my sample tray in the instrument, right, so what I'll wear my sensor, my sensor tray is here, okay, these are the 7 sensor so I'm using, I'm placing here in the sorry, I can't show you this one once, after the experiment you guys can look into that, I will place here, and my sample plate, so we had done, we had probably we had placed all above where we can say go.

So when you say go, we have started experiment the _38:38_ the black, you know, so therefore these are the status, it has quicker _38:54_ first well containing a buffer, nothing is there, this kind of a very noisy data, I think is there 60 seconds to acquire those stats, so as I mentioned we have software we can extend the time as well as _39:22_, so nothing is binding that's why there is no interference changes from the sensor table.

(Refer Slide Time: 39:50)

Unidentified Speaker: Why is this getting _39:56_?

Mr. Susheelendra Vaidya:- Probably the well volume and all that, so now if you look at here, now the binding is happening, the protein A is coating to the streptavidin, as the molecules binding to the sensor surface gradually rising the sensorgram in that. As the molecules bind to the sensor surface you can see the shift in the sensorgram, I had done around 300 seconds I had put for this around by 50 seconds I'm almost, my criteria is more than 0.5 nanometer, between 0.5 to 1 nanometer I should get the loading, that's good enough for me for the interaction studies, I don't want to even more crowd on the sensor surface the immobilization, so even I can go to the next step, I'll do it around 1 nanometer loading then we can study that.

Unidentified Speaker: What's the affinity _41:12_

Mr. Susheelendra Vaidya:- From 10 picomolar to 1 millimolar we can go, that is about un par with your that biacore, so around that's good enough for me I think loading 1 nanometer, okay, fine I'm going to the next step that's good enough for me, the immobilization level.

Next if you look at this columns, green highlighted one the particular step is happening, now if you look at the next step is the baseline where if it is there any unbound molecule just get

washed off in that, and very negligible, the amount is very negligible whatever you're immobilizing, in the well you have so much but when it is bind to sensor surface it's very little, so 60 seconds will be your baseline, it's more stable baseline what I'm observing 30 second is good enough, I can jump to next step, I need a very stable baseline, sometimes what happens is due to, there is a maybe you can observe a drift and all that, so this is very stable baseline.

The drift probably comes from different components in your buffer systems that play important role, when you have a protein you have a stabilizer with some surfactants or some other components, you have a diluted in a different buffer altogether, when you are doing dilution that probably sometimes causes those drifts.

So if you look at now the next step is the association it most to the association, here if you look at I'm aligning the data, this is the first concentration 10 microgram per ML, then the next 10 then the 3.3 microgram per ML, this is your mouse monoclonal antibody binding to the immobilized protein A.

Very simple experiment, so nice those distribution, so I think lower ones are not picking up it is in the sense, it is too diluted the samples or otherwise we had to extend the time, it has to come very nicely those distribution, because we had just put around 120 seconds, probably we had to extend to some more another 60 or 120 seconds, so the lower also will come very nicely.

So let's acquire for 120 seconds, so we are actually I forgot to mention we are working with 1000 RPM, so we have a shaker range working from 100 RPM to 1500 RPM, depending upon the applications we can choose the right RPM. Generally when we are working with the kinetics experiments we go with the 1000 RPM by default, when we do the quantitation experiments we generally work with 400 RPM where we are working with the microgram level concentrations like 200 to 1 microgram in that range, that's fine, when you are working with a concentrations like in kinetics experiments we go with that, so now it move back to the dissociation step, you can observe the bound molecule start dissociating, so just I'm doing around 180 seconds, generally either any SPR or if you take a BLI technologies, so dissociation rate it is the 5% or 5 to 10% dissociation is good enough to calculate the KD value, it is need not like that you have say suppose I'm giving a example you immobilized a 100 molecule on the sensor surface, then you are 100 analyte will bind to the sensor surface, then it is need not like that all 100%, 100 molecule has to dissociate, just 10 molecule if you get dissociation then you typically observes like this that's good enough for you to calculate the kinetic constants as well as the absolute.

Unidentified Speaker: So you say 10% you need, 10% of the signal height, but they comes down by 10% then you are…

Mr. Susheelendra Vaidya:- Yeah, that's another set, good enough.

Unidentified Speaker: So that's enough time.

Mr. Susheelendra Vaidya:- That even SPR also same, so good enough, need not like that we had to dissociate completely all, that's why we go with, there is a condition called as a

regeneration, so rest 90 is bound to your sensor surface right, using a some agents like higher strength solutions like 1 molar or 2 molar sodium chloride or a magnesium chloride or if you want to change the PH like glycine, PH2 or PH1.5, 10 millimolar glycine that's good enough or if we go with the 50 millimolar sodium hydroxide solution, so that will remove the remaining bound analyte, so one thing we had to consider when you are doing the regeneration is stability of your ligand, whatever you had immobilized on the sensor surface that should be stable after the regeneration, there may be a sometimes what happens if you are using a some conditions like glycine or some higher like a base side probably you are quoting undergoes a denaturation, so then next experiment we can't use the sensor back, so that's why we had to carefully consider the regeneration solutions.

When we test the compounds like small molecules generally comes in that range, nanomolar to micromolar range so that time we start with the some 100 microdalton, beyond if you are trying with that concentration is a part of.

Unidentified Speaker: So that's interesting question, with biacore the size of the interact with the analyte I think…

Mr. Susheelendra Vaidya:- Mass change actually.

Unidentified Speaker: The bigger it is the easier just to detect.

Mr. Susheelendra Vaidya:- Yeah.

Unidentified Speaker: Is that _48:04_

Mr. Susheelendra Vaidya:- It's similar, as the low, the size way is the molecule has a very small, then you can see a noisy sensorgrams, even in biacore also same.

Unidentified Speaker: Yeah I know, biacore is definitely that way.

Mr. Susheelendra Vaidya:- Similar, we also seen that, as a bigger molecules monitor sensor surface you get a nice sensorgram.

Unidentified Speaker: Yeah, I see, I see so you know.

Mr. Susheelendra Vaidya:- So I think it is done.

Unidentified Speaker: What's the smallest that you get?

Mr. Susheelendra Vaidya:- 150 dalton.

Unidentified Speaker: 150 dalton.

Mr. Susheelendra Vaidya:- Yeah. So I think now status saying that instrument is ready, experiment is finished, and here also you are seeing that experiment is complete, so we got a data, now we had to go for the data analysis, so what we got the data for using from this? So I

will minimize this, then I will go for the data analysis I'm here, I'll double click on the data analysis, okay, then on my left hand side and the bottom where your data is stored, so here I have a folder called IIT demo, I will drop down here, then I will click on the kinetics, the experiment name I provided is the kinetics, the pink colour file here is just I'll double click on this, when I double click automatically the kinetics data will be opened here, because I started the experiment in a kinetics mode, so we have a quantitation mode if you run a experiment in quantitation mode the data will opened in the quantitation, if it is kinetics it will open in the kinetics, so I'll click on this, okay, this is what the data is to get opened, in the processing section I need to process the data, so what I had to do? I had to subtract the blank from the reference well from the rest of this one, so why I mentioned? Because sometimes buffer also use a artifacts, so we had to substrate those datas.

This is the raw data entire view here, raw data view and alignment and all that if you can particularly you can click on these and this is what the data is, your association and the dissociation, okay, so here in the top section your sensor selection what I had to do is, I had to the subtraction, I had put here 0 concentration what I will do is just I'll select this, right click it, change well type to reference well, okay, then I enable it this, now what I will do is I will go for the subtraction here, in the subtraction I will use a reference wells, we have a different referencings, so we call it as a parallel referencing, then the double referencing and all, so depending upon the experiments sometimes you find a nonspecific binding that time you had to go for their double reference or the parallel reference, but this experiments actually, prior to any experiments when you are going to setup take a sensor, single sensor dip into the analyte, okay, no immobilization, no nothing, when the sensor when you dip into the analyte, your analyte should not interact with the sensor, it indicates that there is a no nonspecific binding, what happens is generally these proteins are the char species, it's a fiber optic, fiber optic is the solenoid O- okay, then you have protein is a positively charged, there is a possibility of the tendency to attack on this glass, then maybe you can see the binding observation, so that's why we add surfactants in that.

But if you are still you are getting a nonspecific binding you had to address that nonspecific binding first and then you had to go for the experiment.

So in this case actually it is a very well tested molecule and IgG's in presence of the tween as well as the BSA it will not show any nonspecific binding, so that's why I went directly with that experiment, so what I enable the reference well subtraction, then they align Y axis, what do you mean by alignment here? I will enable this, align to baseline, what exactly the baseline is here? What exactly it do is, so end of your baseline I need a very stable baseline, end of your baseline last 10 second it will take it and the beginning of your association origin is 0, so data get aligned, I will take it align Y axis to the baseline, I had done the 60 seconds I will take a last 10 seconds of your baseline, so 50 to 60 I will put a 50 to 60, then there is a inter step correction, one more function is there, what exactly inter step correction is nothing but when you are performing the baseline and the dissociation in the same well we can use this function, inter step correction, so what it does? End of your association, beginning of the dissociation that data get aligned, because its work on the dip and rate, sensor move from one well to other well, when it dips there maybe possibilities of the, you can observe a some gems, so those gems has to be aligned, so that's why we use this inter step corrections.

And then sophos web filtering that is for the smoothening function, the sensorgram gets smoothened, so then we say that they process the data, when you process this is your raw data, this is the subtracted data and then when you look it the alignment Y, alignment Y as I mentioned end of your baseline, beginning of the association then the align axis data is nothing but end of your association, beginning of the dissociation to get aligned, so this is, if this is the perfect sensorgram, so we had done the processing of the sensorgram, now we have to do the analysis.

The next window is your analysis here, in the analysis we have fitting models, we have different fitting models, 1:1 fitting model, 2:1 fitting model, 1:2 heterogeneous, there are different binding models are there, so generally when you do any bio-molecular interactions we try to fit for 1:1, 1 protein binding to the corresponding 1, there may be a some situation in the biological process, there may be a 2:1 heterogeneous binding model or 1:2 bivalent models and all that, so we had to choose the right one.

If you know thoroughly because when you are doing a publication say suppose I got a 1:1 fitting data, that was not giving a good fit, but you go, you went for the 2:1 fitting model, then you submitted a minus script to the some general, then the reviewer will ask you one question, you have mentioned the fitting 2:1 binding model, do you have any supporting data by any as a technique, it is a 2:1 interaction, you have to provide, generally reviewer ask this question for any peoples, so that's why we always try to fit for a 1:1 interaction, otherwise if you have a ITC data with a 2:1 stoichiometry, then that's good enough, or otherwise you had to establish that, or otherwise you had to modify the protocol where you get a 1:1 interaction.

So then we have a fitting models I had chosen a 1:1 binding model, and then fitting, in the fitting what his local fitting and the global fitting, local fitting is nothing but the calculating the K on, K off as well as the KD values correspondence to each binding curves, sensorgrams, each concentration, it fit each one, when it go for the global fitting it will consider the mean of your calculation all the binding curves, generally when you're publishing a data or people prefer for the global fitting data rather than the local fitting, so we will go with a global fitting, so what we do is global fitting is I'll choose here, because each sensorgram also giving a 1, 1 colour, but I want to make it into 1 colour in a 1 group, so what I'll do is I'll select here right click it, set colour by, I will choose 1 blue colour here this and I will say go, if you look at all the sensorgrams become blue colour, it will become a 1 group kind of thing, so what I will do is global fit group by colour I'm using and R-max unlinked by sensor, I'm choosing the R-max unlinked by, there is a experimental design R-max linked and R-max unlinked.

R-max linked when we are doing is, suppose I'm taking a one sensor and passing the all the concentration on to the particular sensor, then we call it as the R-max linked, because I had taken a 6 concentration, all the 6 concentration you have passed on the one sensor that is R-max linked, but my experiment here in the high-throughput what I'm doing is, 6 sensor or 7 sensors I had taken and each concentration I probed with a 1, 1 sensor, that's why I'm unlinking that, so I'm using R-max unlinked, so this is then say fit curve, okay, when you do the fit curve, if you look at this, this is the association, the red one is your theoretical and the blue one is your experimental, so if you go here this then you can get the tabular column, you have a KD, it is

KD is around 3.7 nanomolar, and your K arm 4.28 x 10 to the power of 5, and the K dissociation 1.5 x 10 to the power of -3, so we can say the how my fit is good, we have a statistical parameter also in that, my fit is good or not, my acceptancy is something like that, if my asper the regression coefficient for the fitting, if I'm getting more than 0.95 then it is a good fitting.

I'm getting around 0.99, it is a good fit, and how significant is my fit is? It's a goodness of the fit and significant of the fit, there are two parameters, we have one more statistical parameter called as the chi square, my chi square should be less than 3, I'm getting around 0.12, so the fit is good, so when your R square is more than 0.95, automatically your chi square also comes down, if it is the fitting is not good then you can see the your difference in the chi square as well as the R square, so this is about the data, or you wanted to look it into how my each concentration if you want to exclude something concentration because I have run 6 concentration, 4 or 5 concentration is good enough for the publication, so if I look at this is, this data if I look at this is not good, so I can exclude this, just a check, this is okay, so one concentration I'm excluding still I can do the fitting.

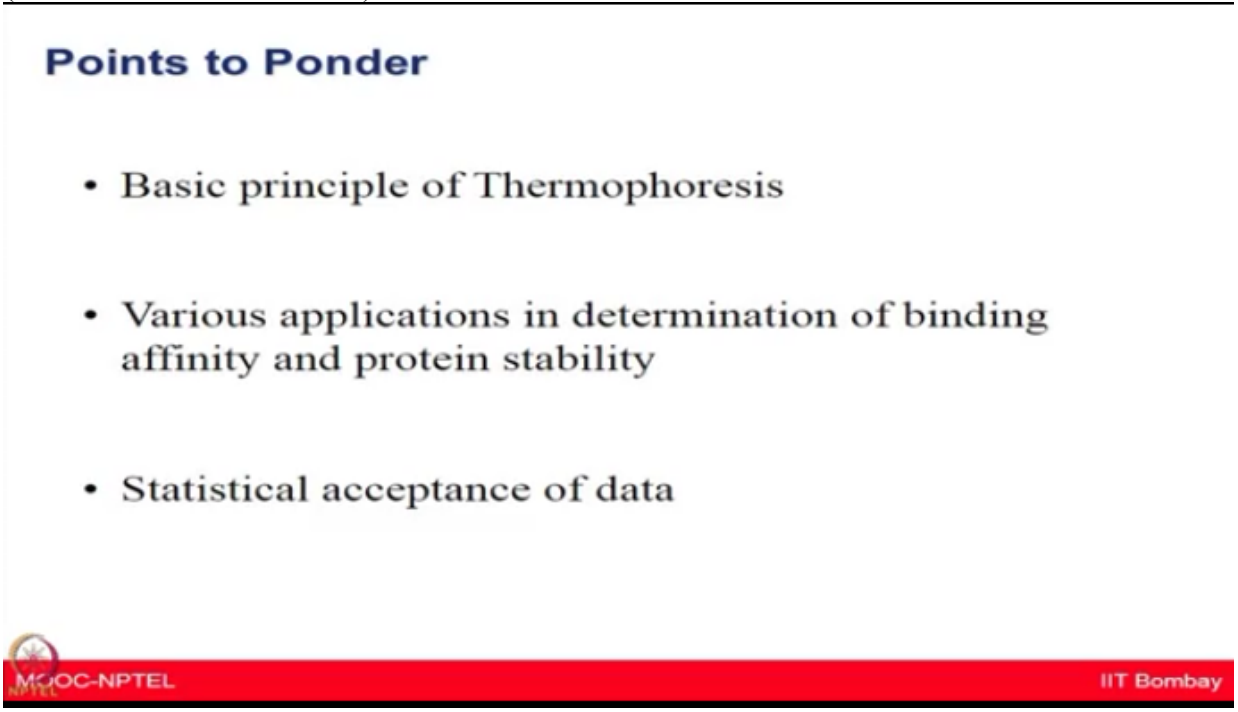
KD is not changed much, but R square somewhat change, so then what I'll do is I will save report, when I save report export, you have a excel sheet report, you have experimental summary here, sensor tray where you had placed your sensor, then processing parameters how we had done the processing of the data, sensor data where you had kept the sensor, sample data how we had, where you had place the samples and all that in the plate, this is your raw data, then the processing data, this is the raw data, subtracted data, align Y, align X, all this data parameters, then the started graph, this is the fitting model and this is the residual view, residual view is nothing but the difference between your experimental and the theoretical, how the residual fit is, that is one, and the results table where the table you have a KD calculations, errors, K-on, K-off, statistical parameters all informations you can find here in this, okay, then you have a iso-affinity graph, iso-affinity graphs play a very important role when you are performing a screen kind of experiments where it will plot a K dissociation versus K-on, so this is suppose giving a example you have a 10 batches of some proteins, if your all 10 batches are behaving similar, then all data points will be coming to the same cluster then you can say that it is on par, everything is passing, if it is some of the batches are something following that side and this side it is deviating from that, this is very important when you do industry kind of a platform or when you are doing a validation or such kind of experiments.

Study state analysis is there one more, but this experiments it is not reaching study states, I will tell you because protein A have a probability of binding a 4 IgG's, one protein A can take up a 4 IgG, that's why it never reaches the equilibrium or otherwise I have to load very less, just a 0.1 nanometer or a 2 nanometer then I can observe the study state, so that also, study state is a very important where study state when happens is your A + B = AB complex rate, 50% A+B is complex formation, and also this complex simultaneously dissociates back to the A and B, so this phase is called as the equilibrium, so such kind of datas we go with a study state analysis.

And the group view, this if you want, if you are performing some other kind of a presentations and or I think different batches when you are running you can get the sensorgrams those things, so these are the report format and say the good advantage is like that you want to, this fitted

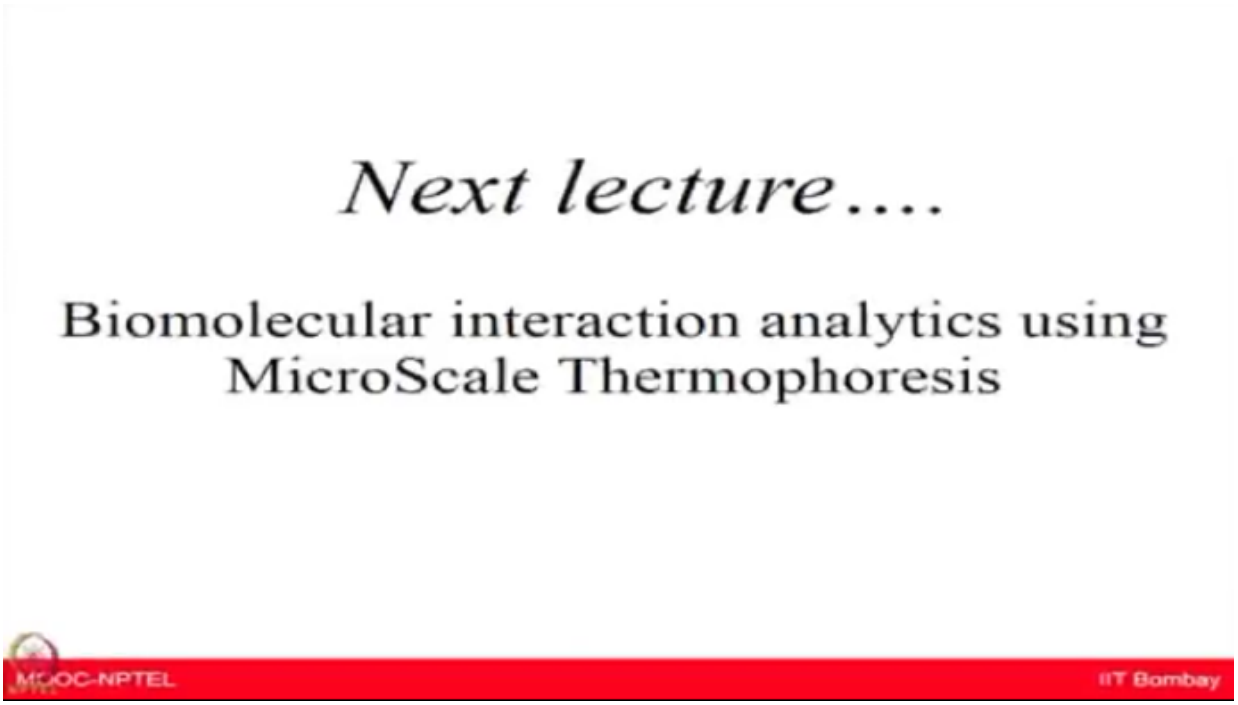
results you want to use for the some other software, like say suppose you want, this data you want to review in a, or analyze in the biacore software, yes, it's possible, or you want to use a scrubber third party software, yes you can do using this, there are multiple options are there, very easy, very quickly you can determine the kinetics on rates and the off rates.

And you have fitting different models are here, 1:1, 2:1, mass transport we have, bivalent analytes we have, different models are there, so this is what about the kinetics and quickly hardly we had not taken a much time around roughly less than 1 hour we finished experiment.
(Refer Slide Time: 01:04:00)



Dr. Sanjeeva Srivastava:-So this now completes the two lectures on bio-layer interferometry for studying bio-molecular interactions where we discuss this with application scientist Mr. Susheel Vaidya from Pall Life Sciences, and he gave you some basic understanding as well as hands on demonstration session of this novel technology platform.

We'll continue discussion about new technology platforms for being bio-molecular interactions, in the next lecture also we'll continue with another technology platform for bio-molecular interaction and let its using microscale thermophoresis or MST technology along with Tycho anti-technology, so these new technology platforms will be discussed in next lecture. Thank you.
(Refer Slide Time: 01:04:56)



Prof. Sridhar Iyer

NPTEL Principal Investigator
&
Head CDEEP, IIT Bombay

Tushar R. Deshpande
Sr. Project Technical Assistant

Amin B. Shaikh
Sr. Project Technical Assistant

Vijay A. Kedare
Project Technical Assistant

Ravi. D Paswan
Project Attendant

Teaching Assistants

Apoorva Venkatesh

Nikita Gahoi

Shalini Aggarwal

Bharati Sakpal
Project Manager

Bharati Sarang
Project Research Associate

Nisha Thakur

Vinayak Raut

Sr. Project Technical Assistant **Project Assistant**