Interactomics: Basics and Applications

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Lecture - 34
Protein-protein Interaction Study: Binding Analysis

To completely understand the function of a Protein, it is important to look beyond its expression, pattern and identify its potential interacting partners and determine the interaction dynamics because most of the systems are not only governed with a specific molecule, but rather how their entire interaction network is built.

To study the interaction between two binding partners in the SPR experiment, one partner is attached to the surface and other is passed over the surface in a continuous flow. The interaction of ligand and analyte is measured by the SPR instrument as a change in reflective index over time and response observed is directly proportional to the change in mass concentration close to the surface.

In the previous lecture, we have performed a lab session and completed the immobilization of anti-beta 2 microglobulin antibody on the chip surface of CM 5 chip. We will now move forward and perform the binding analysis of anti-beta 2 microglobulin with beta 2 microglobulin protein. As I mentioned we are talking to you about a standard protein an antibody pair. However, the same steps, same procedures could also be followed for protein of your interest. However, if you want to design considerations may have to be modify it as per the protein and antibody pre-requirements.

Let us continue our lab session and watch how to perform the binding analysis of anti-beta 2 microglobulin with its protein in the lab setting.

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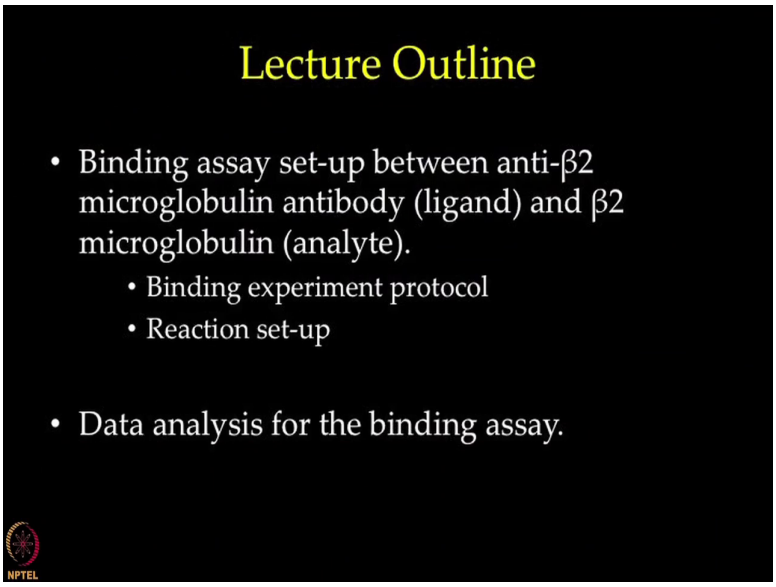

We will go into the second session now and we will use our immobilized chip for a binding experiment.

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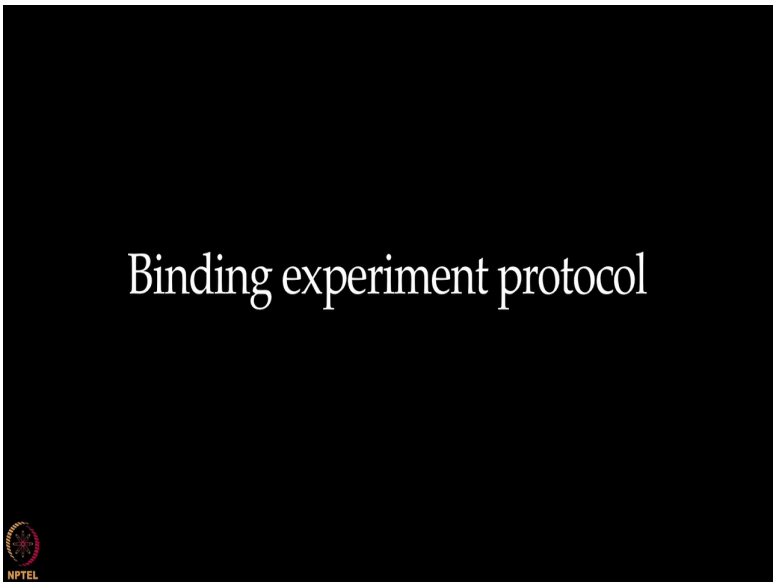

So, here we will first prepare a template for the binding experiment.

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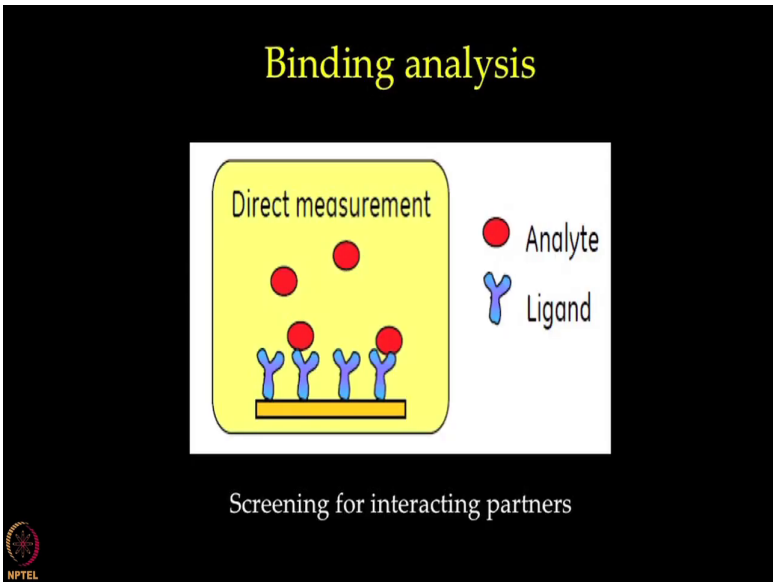

A binding experiment actually deals with screening or a single concentration screen of different compounds on the immobilized ligand and look for its ability to interact with the target that is immobilized on the surface.

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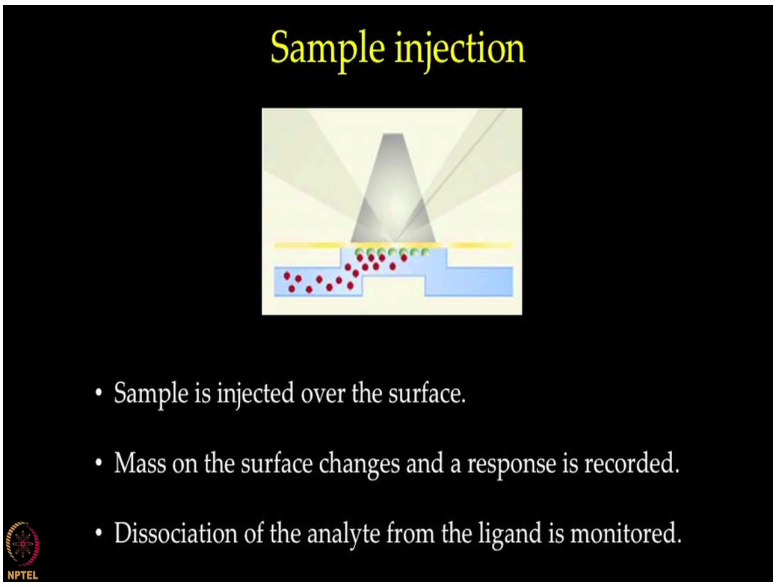

Before we go ahead with the binding experiment let us understand some important considerations. During sample injection the analyte is injected over the surface with a constant flow in concentration. Analyte in the sample binds to the immobilized ligand on the surface, the mass on the surface changes and the response is recorded. After sample injection buffer flows over the surface to allow monitoring of analyte disassociation from the ligand.

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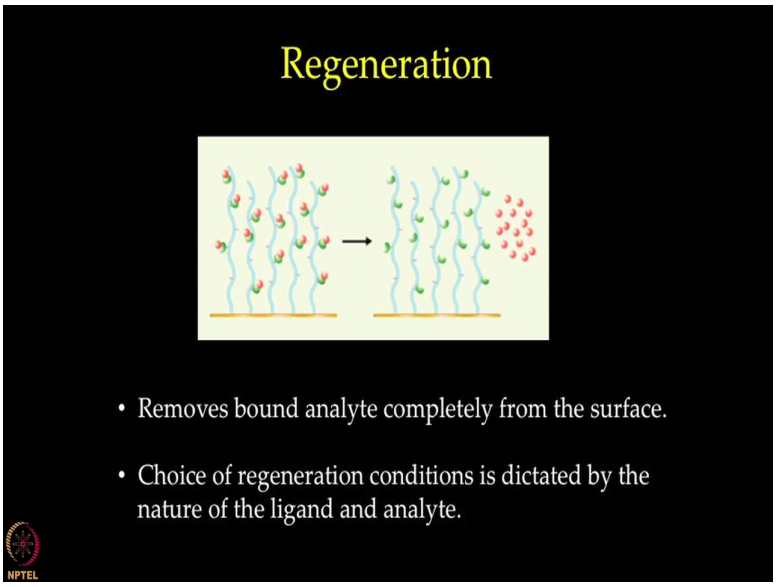

Regeneration as already discussed by Doctor Srinivas is the process of removing bound analyte from the ligand on the sensor chip surface after analysis of a sample. Efficient regeneration which means removing bound analytes without affecting the ligand activity is crucial to a successful assay. If the regeneration is incomplete or the binding activity of the surface is reduced, the performance of the assay is impede. The choice of conditions for regeneration is dictated by disstability and nature of the ligand and analyte.

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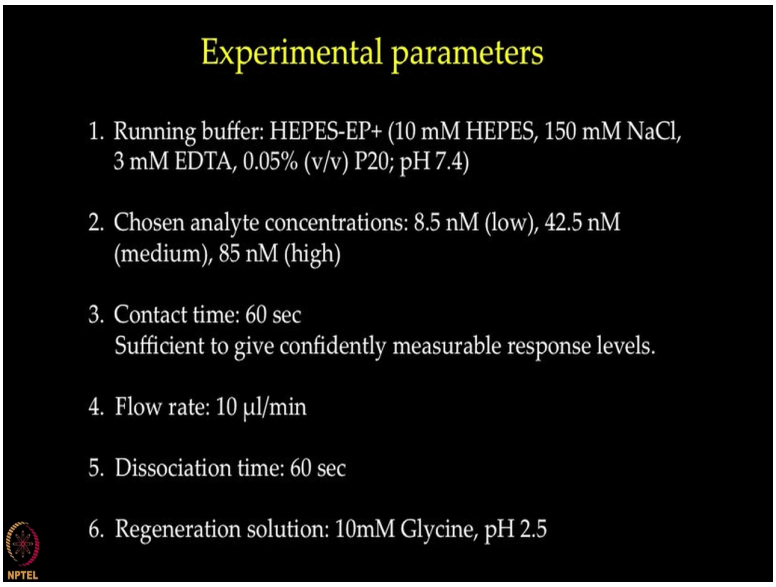

In today's binding experimental setup we will be using HEPES EP plus as the running buffer. We will be preparing 3 different concentrations of beta 2 microglobulin protein which are 8.5 nanomolar, 42.5 nanomolar, and 85 nanomolar for evaluating its binding with the antibody having 8.5 nanomolar concentration in duplicate. These 3 concentrations will be referred to as low, medium and high.

Contact time between the sample and the sensor surface should be sufficient to give confidently measurable response levels without compromising screening throughput. Contact time of 1 to 2 minutes are usually sufficient for a binding experiment Here we will provide a contact time of 60 seconds as the flow rate of 10 microliter per minute with the disassociation time of 60 seconds. An ideal regeneration condition is the one that analyte response of the same concentration is constant after repeated injections.

Today we will be using 10 millimolar glycine pH 2.5 for regeneration of the surface. We will now proceed with our binding experiment protocol.

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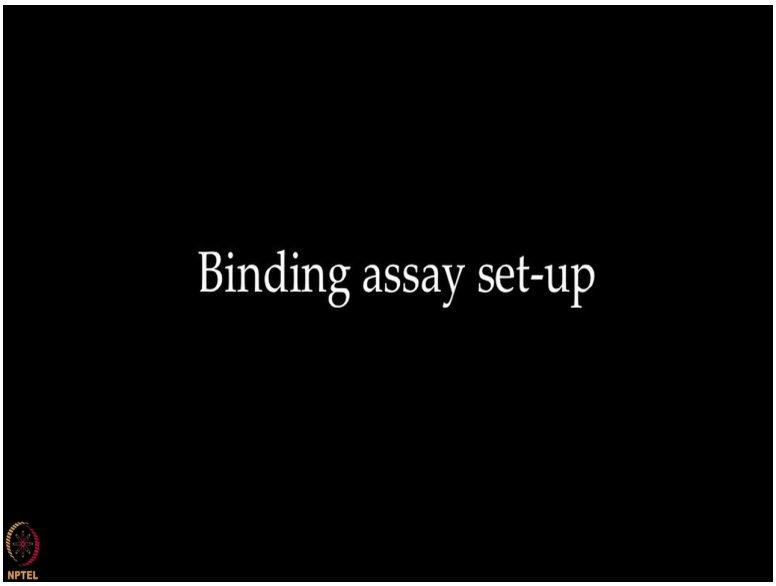

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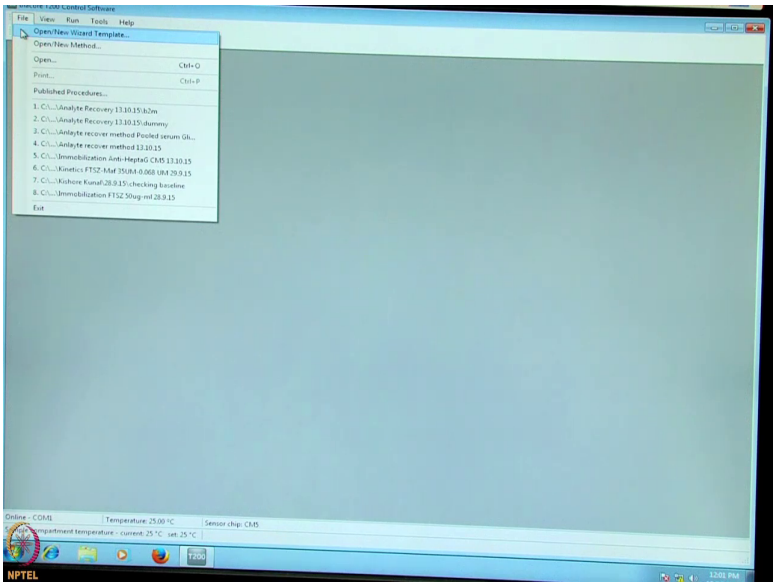

File, before making the template we will open the file wizard again.

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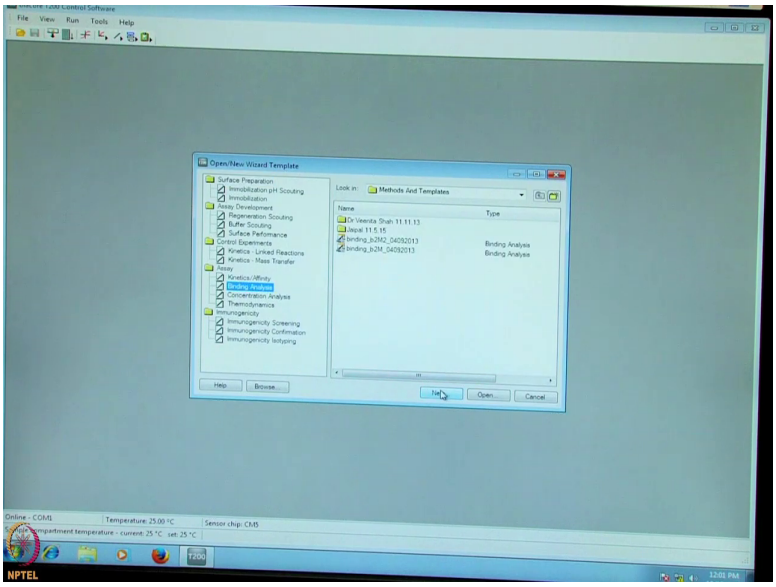

Binding analysis. New.

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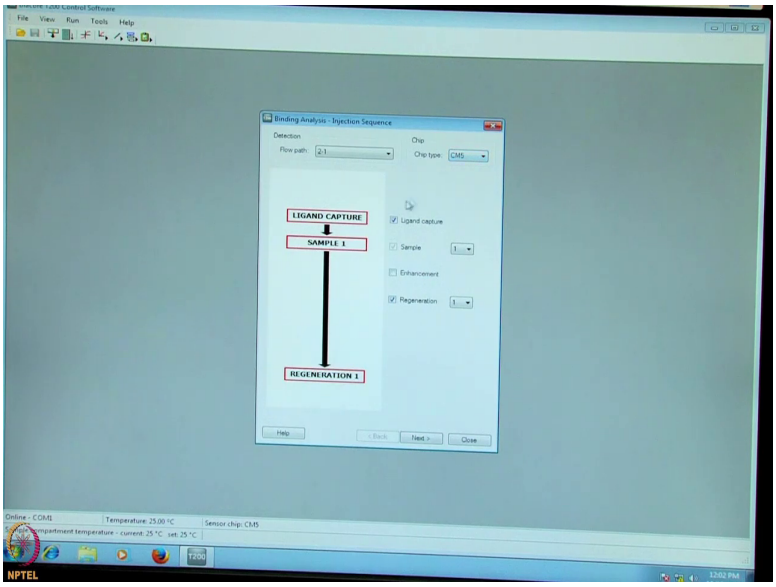

Identify the flow path as 2 minus 1 as we have done our immobilization on 2 minus 1. Chip will be CM 5 that is blocked already.

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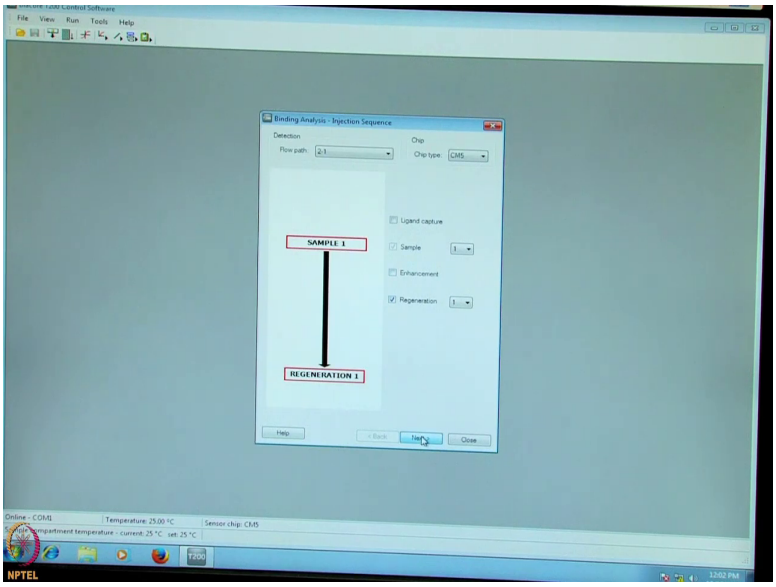

We will not have ligand capture. Sample and regeneration. We will go to the next tab.

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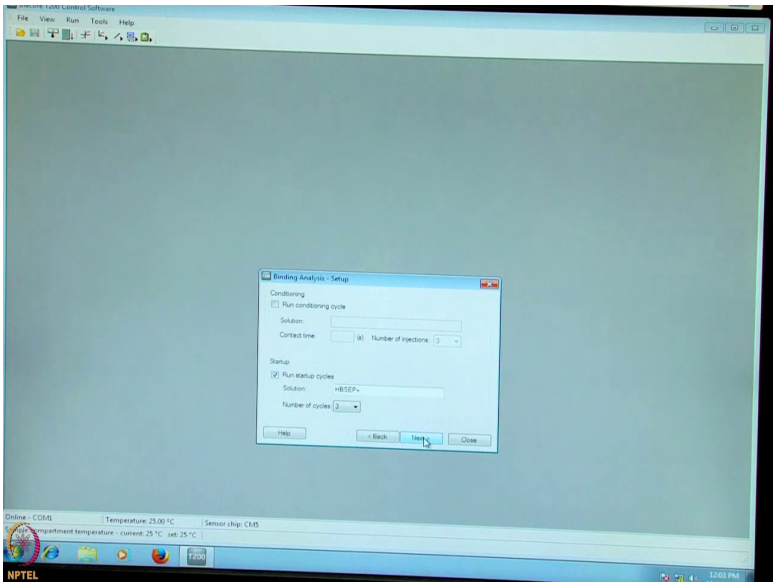

Here we are not using any conditioning cycle. We will start with the startup cycle. Startup cycles are cycles of buffer used for equilibrating the system. So, here basically buffer is used as analytes. So, we can type as HBSEP plus buffer and from the pull down menu we select 3 cycles. Generally, for binding experiments 3 cycles are selected. Going to the next tab for setting up a binding.

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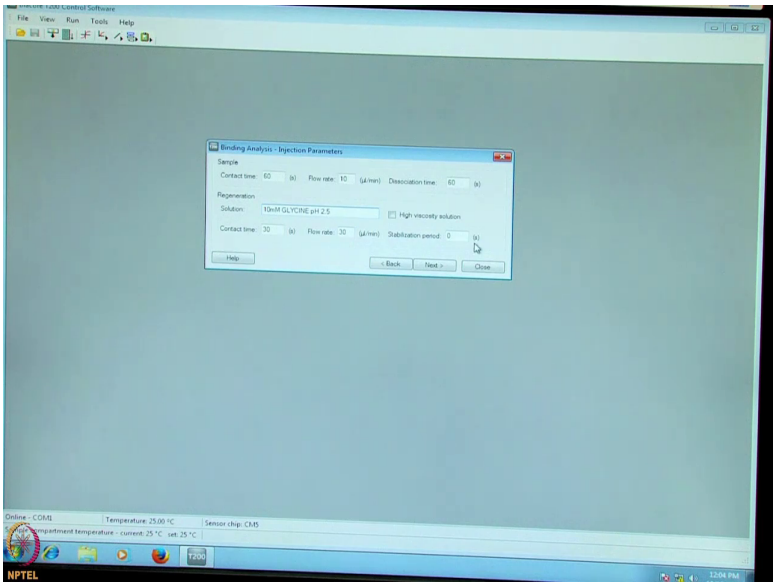

We need to specify the contact time as 60 seconds default. Flow rate of 10 microliter per minute. Disassociation time of any minute or any second, by default we could consider 60 seconds. The regeneration solution we would prefer here would be 10 millimolar glycine pH 2.5 with the default contact time of 30 seconds, flow rate of 30 microliter per minute and with no stabilization time.

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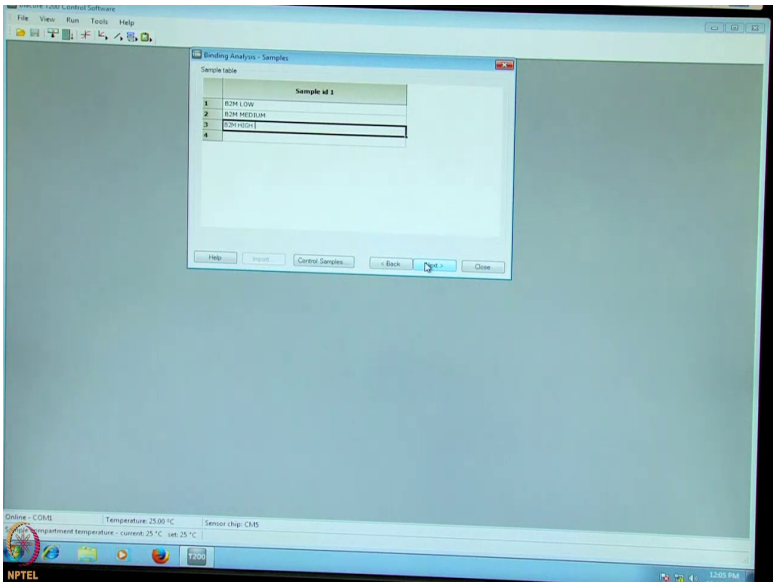

We go to the next tab. Here we need to fill in the name of all single concentration compounds. So, here we would select our analyte as beta 2M. Just that we have one analyte we will take it in 3 different concentrations. So, we will name as low beta 2M medium and beta 2M high. So, low indicates lower concentration, medium concentration, high concentration.

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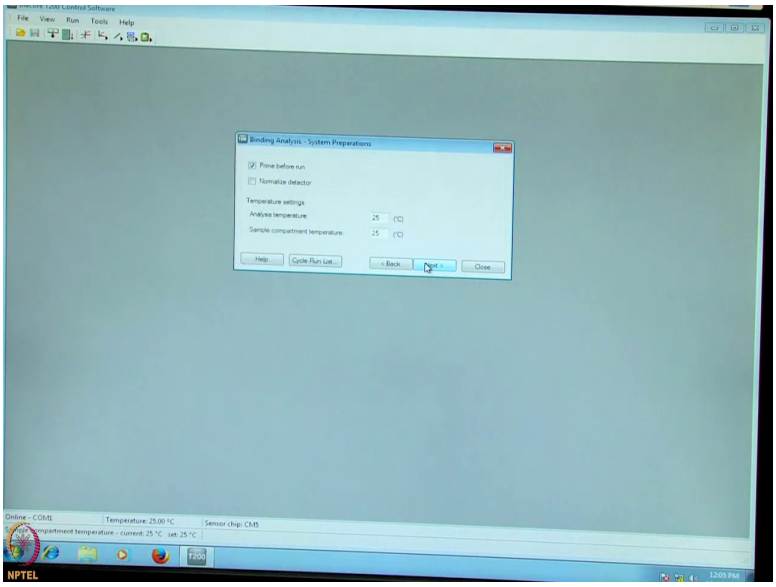

And we will go to the next tab, we will select prime before run and normalize is not required here because the chip is already immobilized and we will go with the default temperatures.

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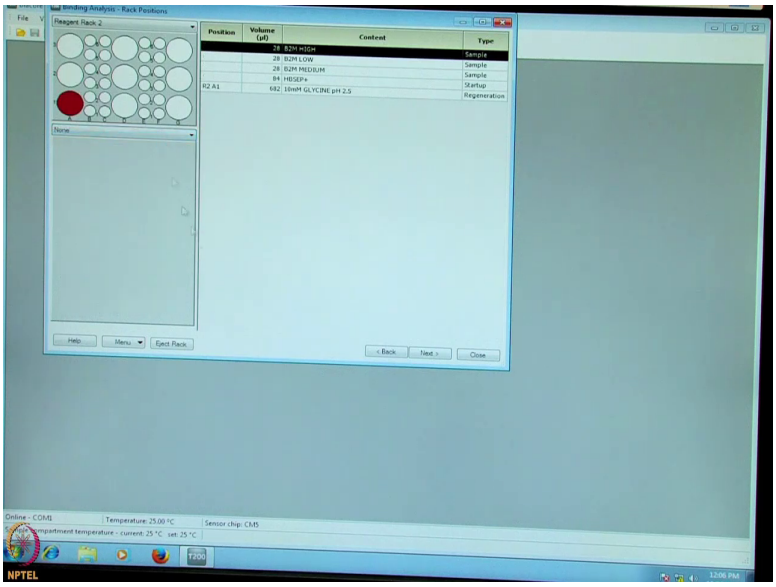

And we will go to the next tab. Here we will not select a microtiter plate. And this is our rack positions for a binding experiment.

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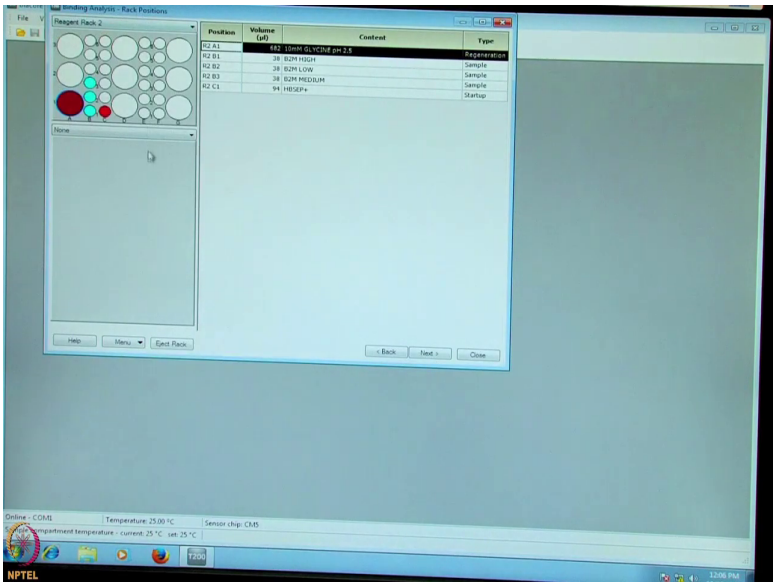

Here we have at the C one position HBSEP plus buffer for 3 different startups. We have 3 concentrations of analyte, high, medium, and low, and we have the regeneration solution here which is 10 millimolar glycine pH 2.5. And we will prepare our solutions and start the binding experiment.

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We will now work under reagents requires for the binding analysis of anti-beta 2 microglobulin with beta 2 microglobulin protein.

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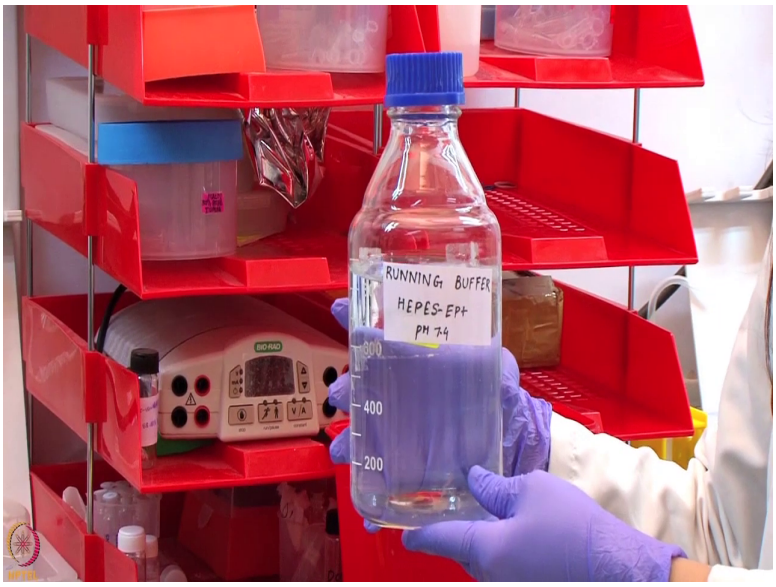

We will be using HEPES EP plus as the running buffer which is also been used for the initial startup cycles.

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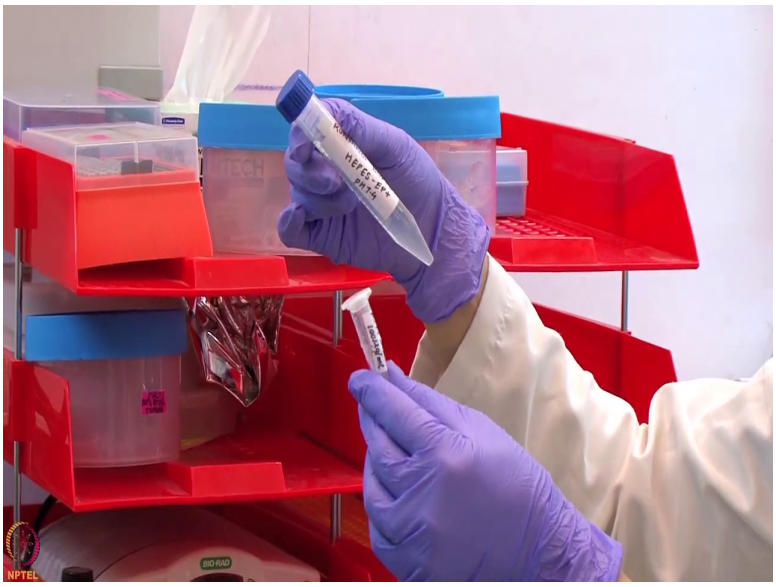

We then dilute the stock solution of protein that is 100 microgram per ml in the running buffer HEPES EP plus.

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To prepare 100 micrometer of 3 different concentrations that is 85 nanomolar, 42.5 nanomolar and 8.5 nanomolar.

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These are referred to as high medium and low concentrations in the experiment.

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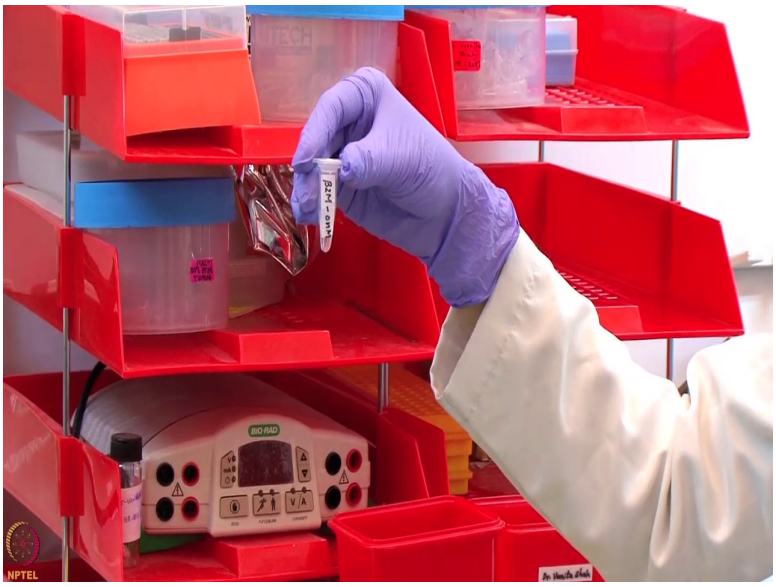

We will also include one 0 nanomolar concentration in the experiment which will be nothing, but the running buffer.

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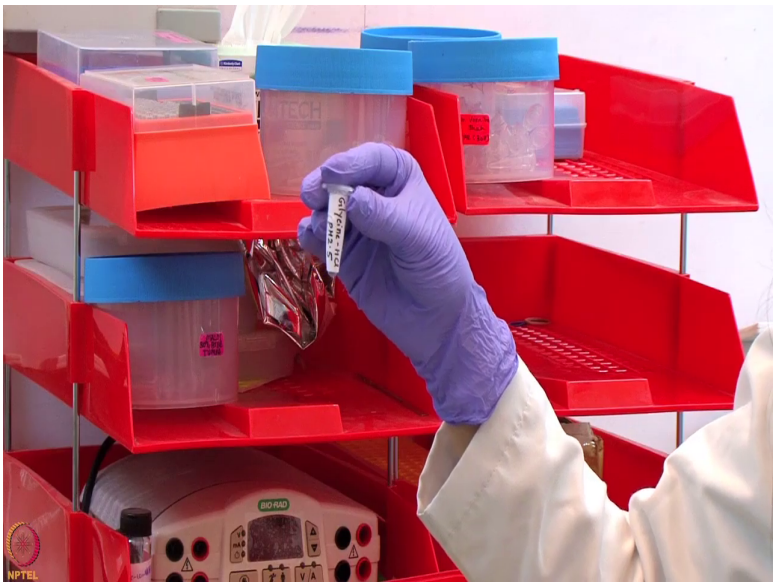

For the regeneration of the surface we have prepared glycine SPL pH 2.5 as the regeneration solution.

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We have transferred all the solutions in the specialized tube starting from the startup.

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Beta 2M concentration starting from 85 nanomolar.

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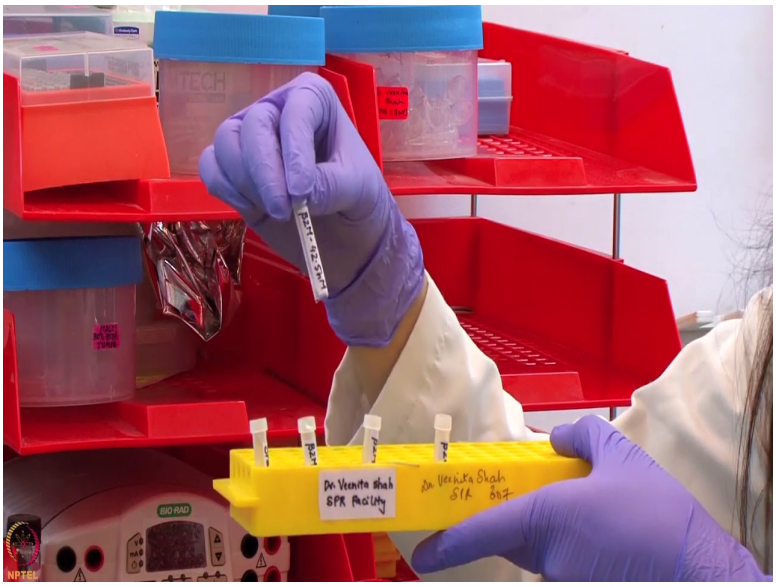

42.5 nanomolar.

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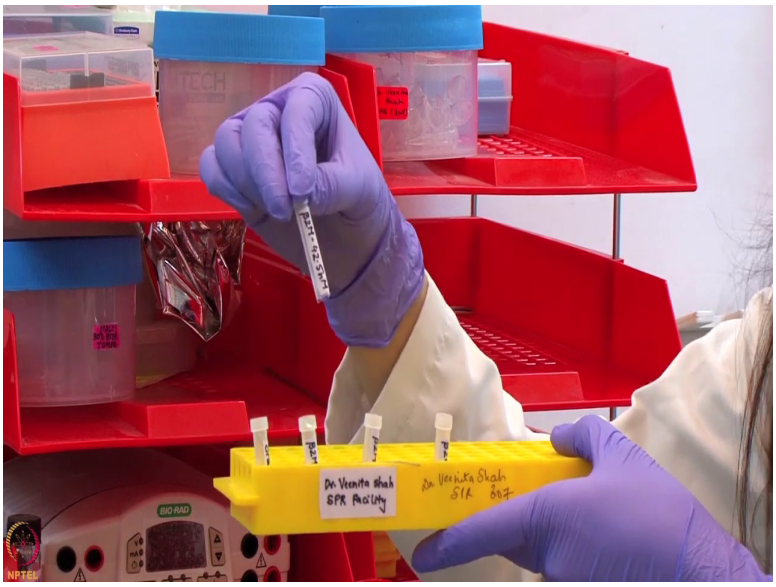

8.5 nanomolar.

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And 0 nanomolar.

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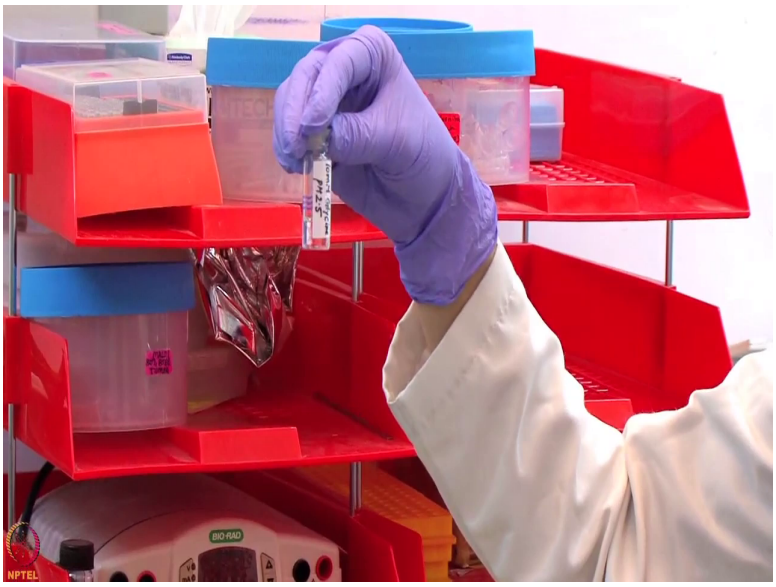

The regeneration solution is placed in this glass vial.

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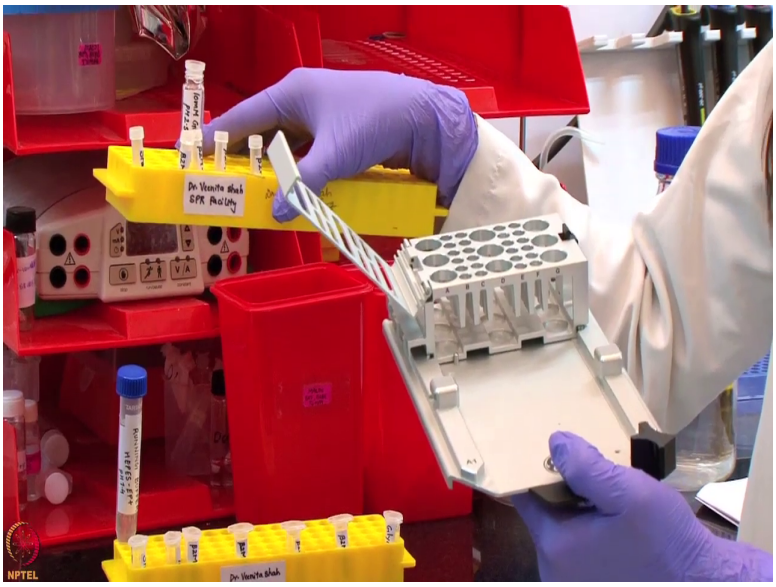

We will now insert these tubes into the appropriate racks and then into the system to start with the binding analysis of anti-beta 2 microglobulin with beta 2 microglobulin protein.

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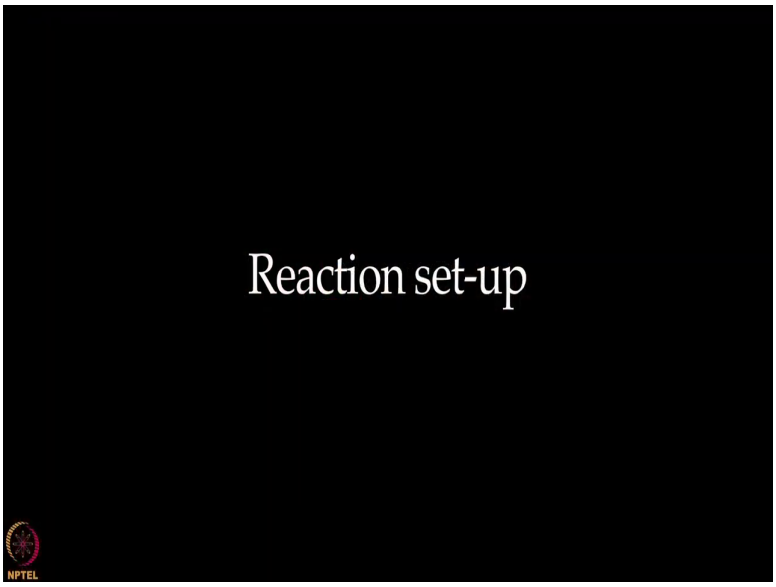

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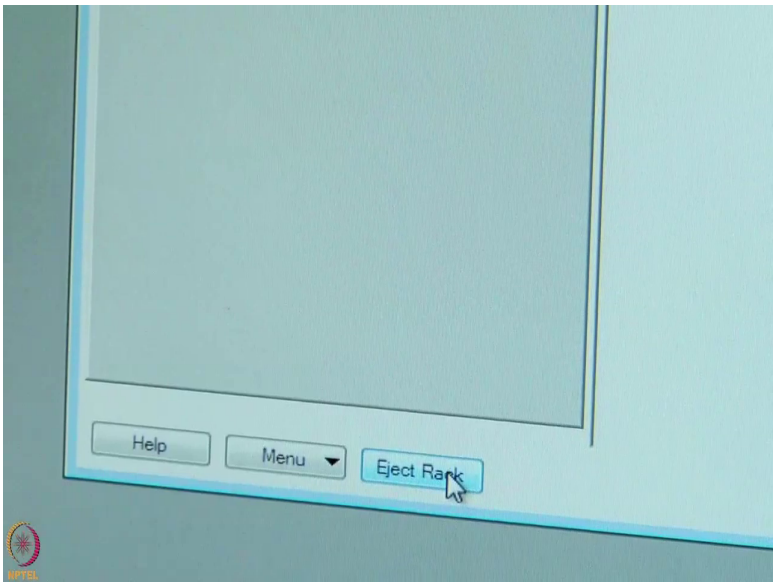

We will eject the rack now to insert new vials, eject rack.

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And take the plate out of the sample rack.

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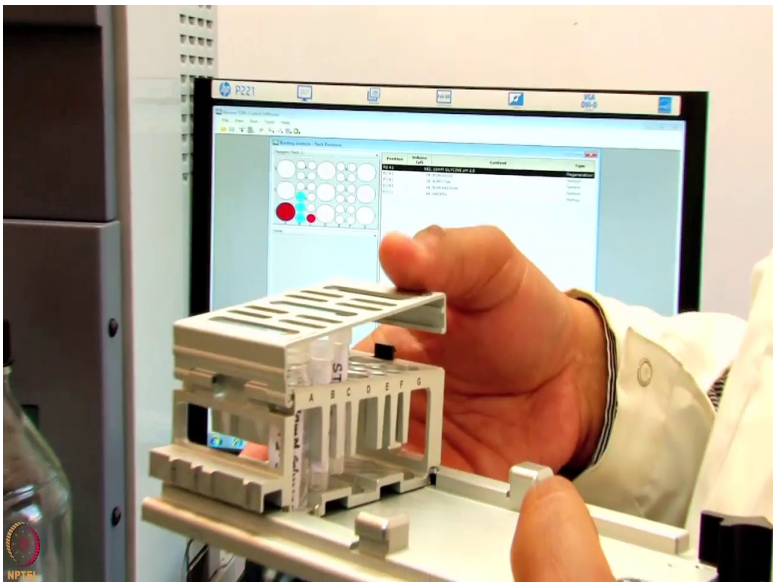

And we will fill it with the binding vials. So, these vial positions are filled with different samples. As you can see on the screen the startup is here. So, beta 2M medium, beta 2M low, beta 2M high are put at their respective positions and a vial for regeneration of 10 millimolar glycine pH 2.5 is here. Now, we close the rack and will be inserted in the sample compartment by ejecting the rack compartment, inserting the plate. Next tab.

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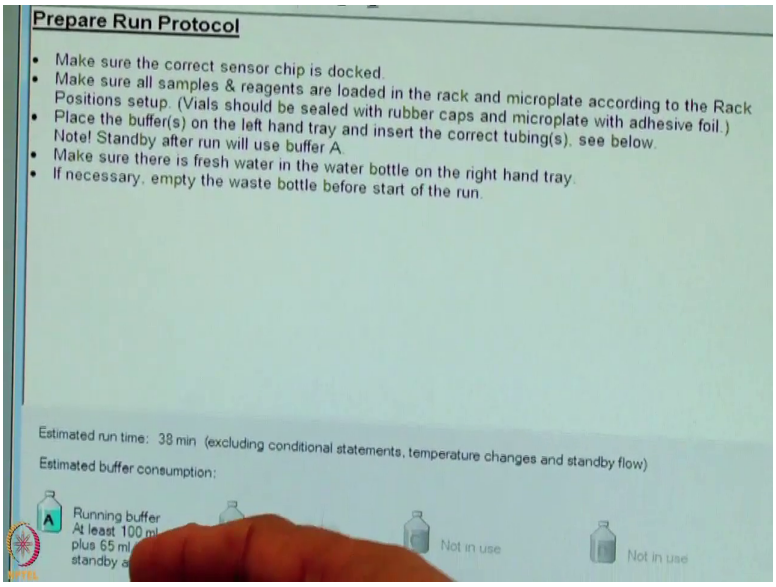

So, again we need to do all these steps, set the time, the estimated runtime of 38 minutes and we have sufficient amount of buffer.

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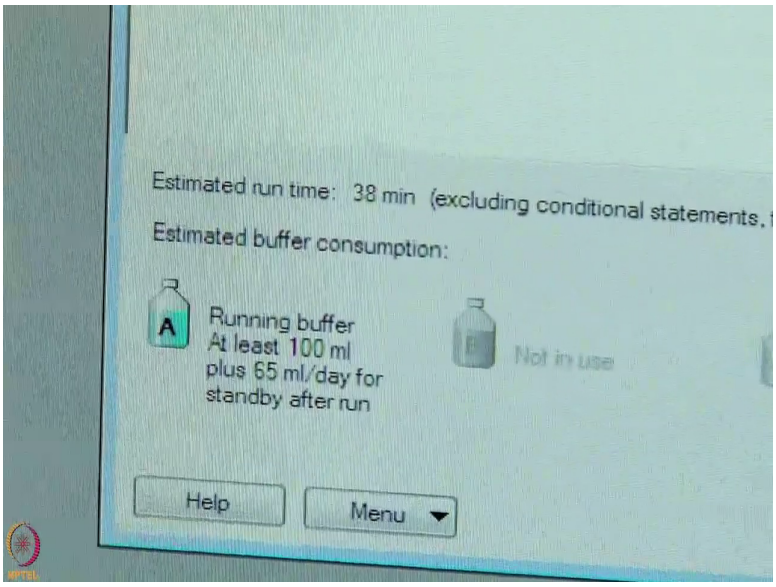

And we will now start the experiment.

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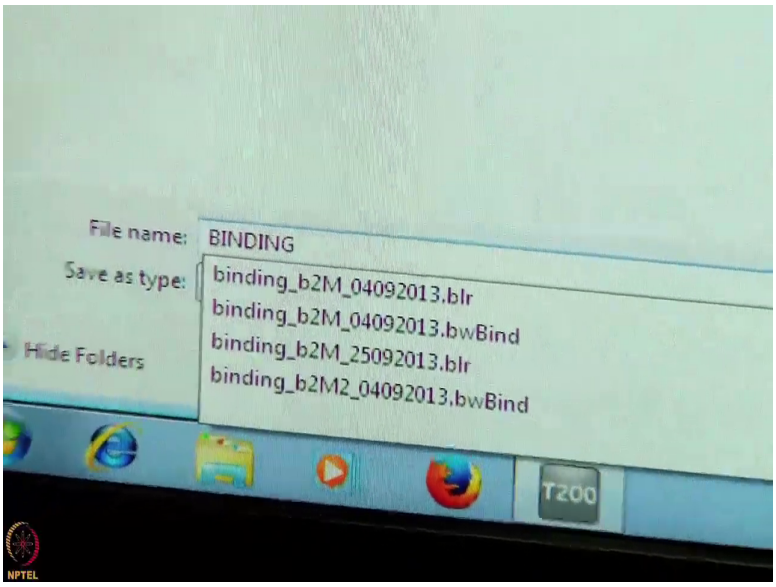

We will save this template as binding, save.

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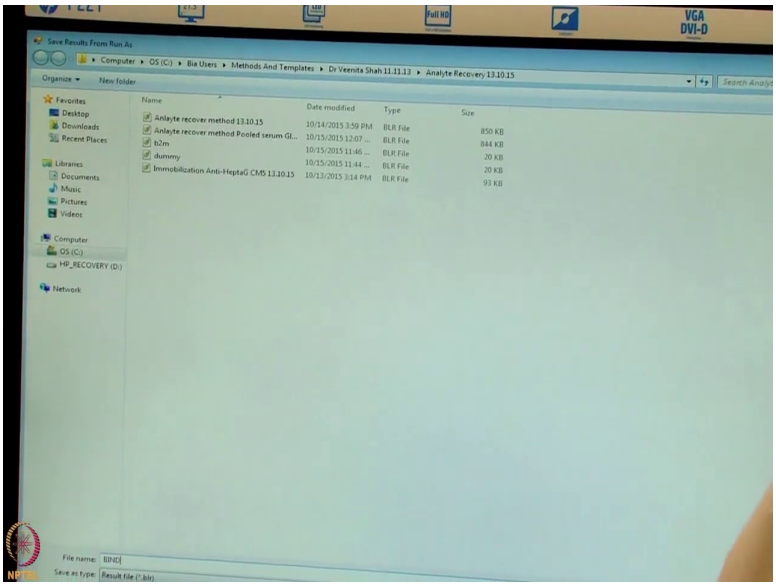

Now, we will save the result file again as binding.

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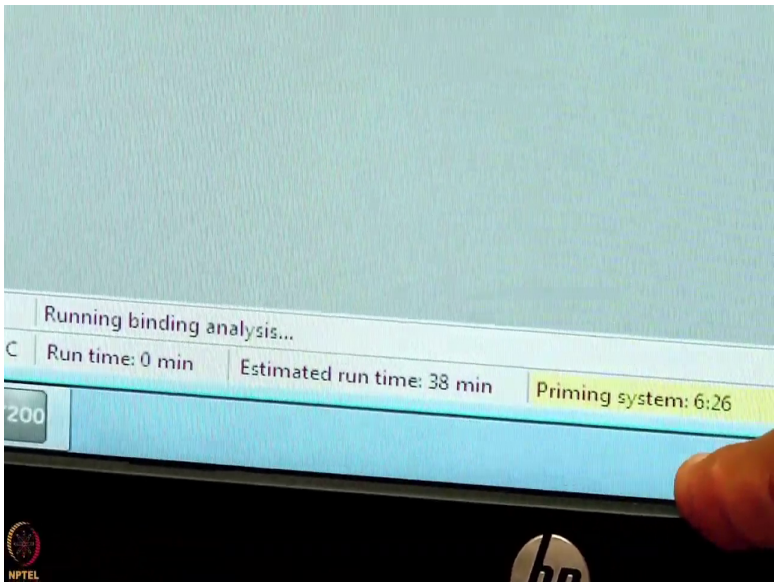

And now the experiment has started shows running binding analysis with an estimated time of 38 minutes. System is priming now and once we finish of the binding experiment we will take a look at the data.

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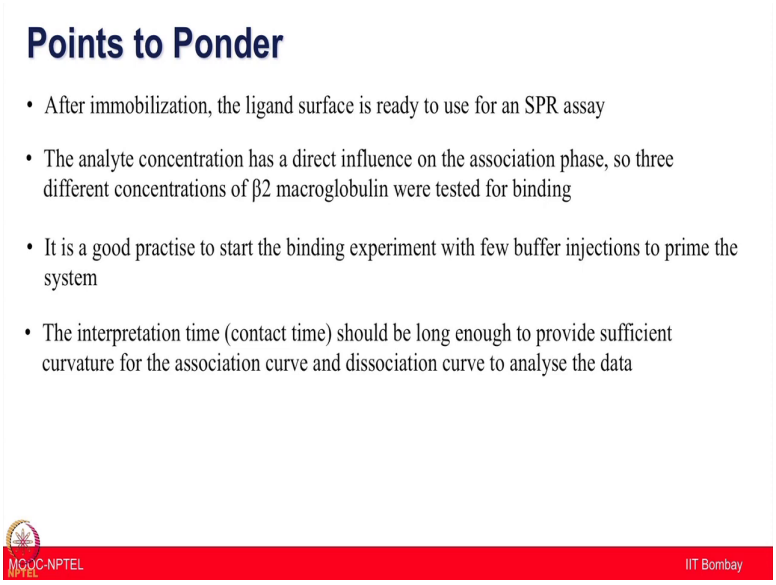

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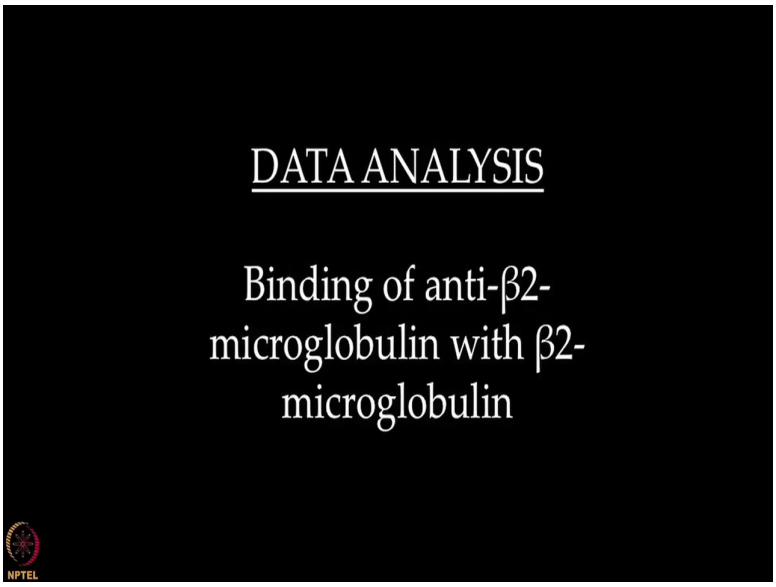

Before we analyze the binding data from the experiment let us look at a typical sensorgram for binding between a ligand and an analyte.

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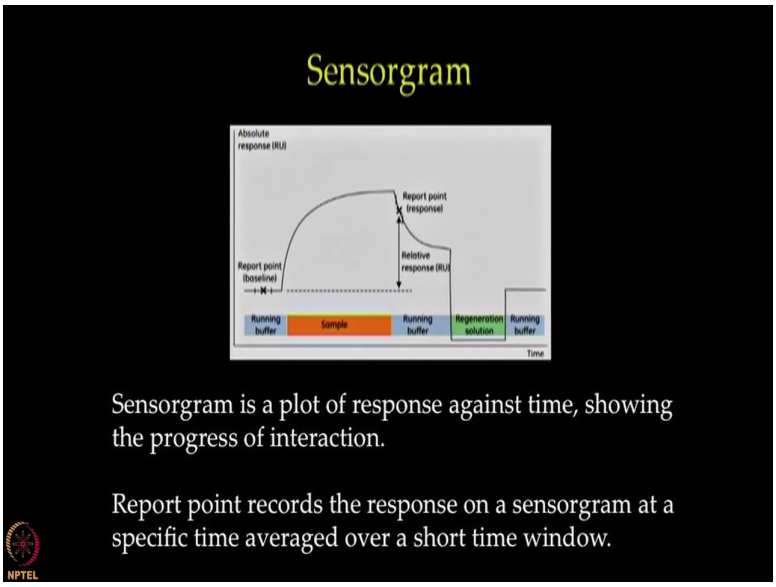

A sensorgram as shown here is a plot of response against time showing the progress of interaction. This curve is displayed on the system during the course of experiment. We observe the base lines followed by the injection of analyte which leads to increase in the binding response during the association phase.

Just after the stop of the sample injection we observe report point which we call the response on a sensorgram at a specific time averaged over a short time window. This is followed by disassociation phase, regeneration and then back to baseline. We will now proceed to analyze the data obtained from binding of anti-beta 2 microglobulin with beta 2 microglobulin protein

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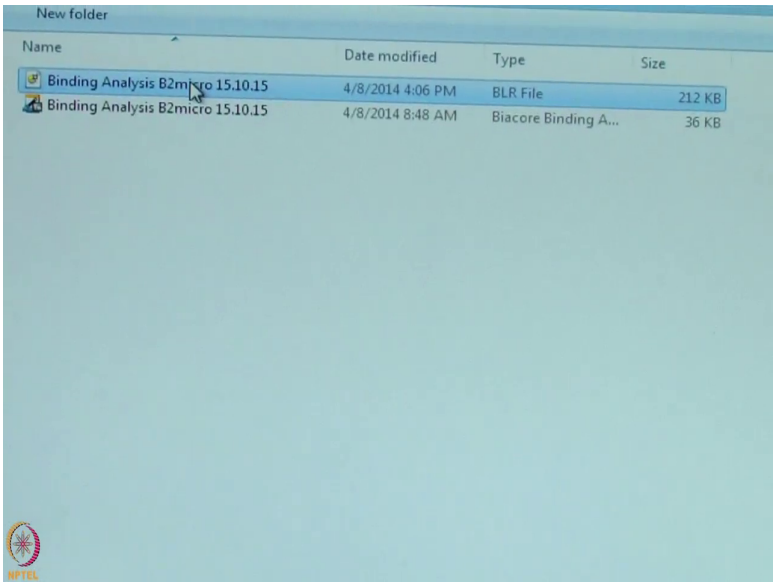

After finish of our binding experiment by double click on the file.

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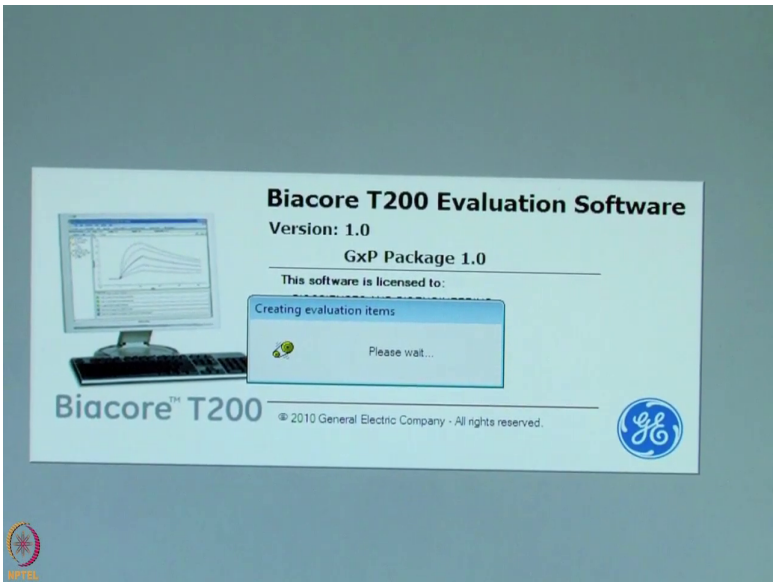

The file is open now.

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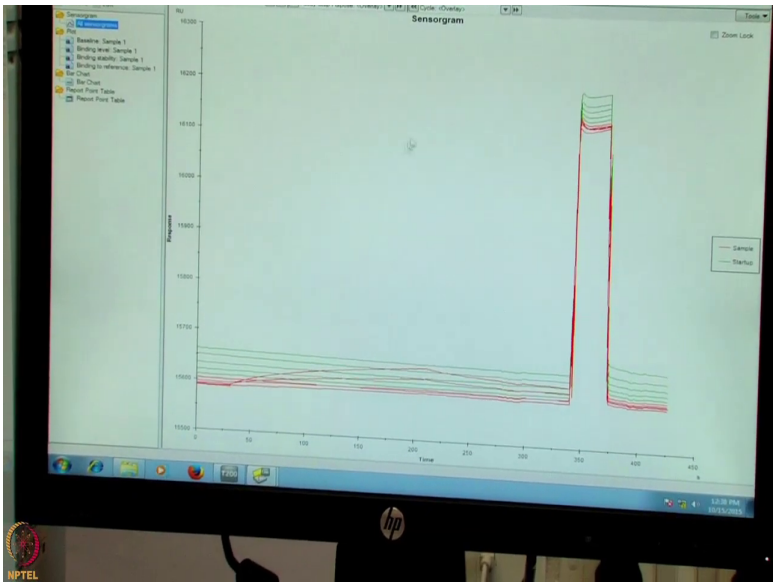

You can see here from our binding experiment which shows all sensorgrams here. So, here the green ones are our startups. So, we have setup 3 or 5 different startups, and the red ones are our actual data from beta 2 microglobulin.

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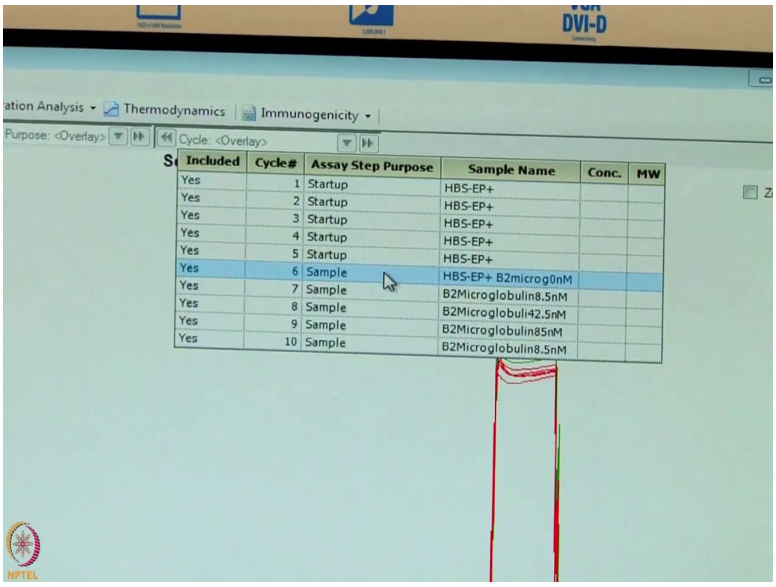

What we will do is we will highlight only our sample data.

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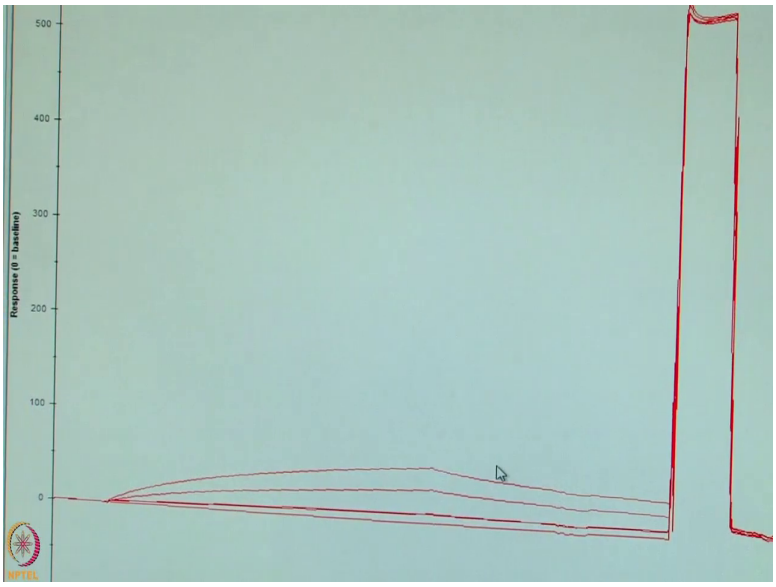

And now you see the data for beta 2M.

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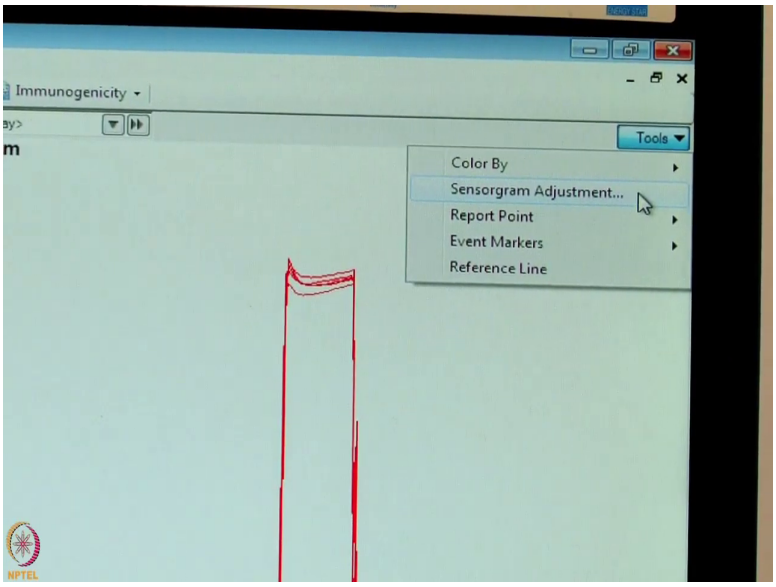
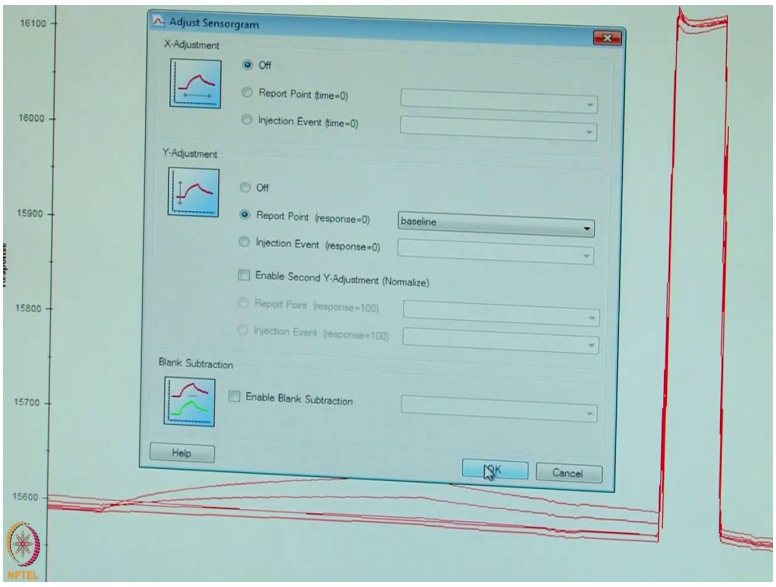


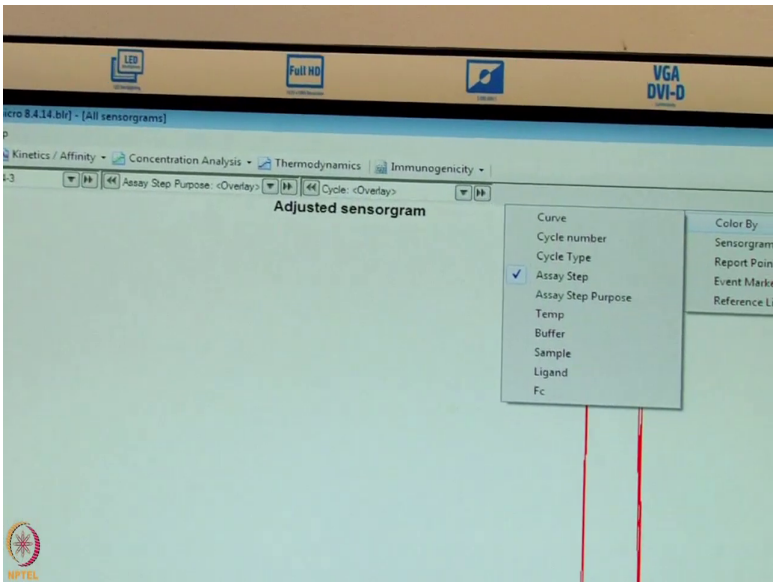
We go on tool sensorgram adjustment to report point on the y adjustment, baseline and say ok.

(Refer Slide Time: 21:37)



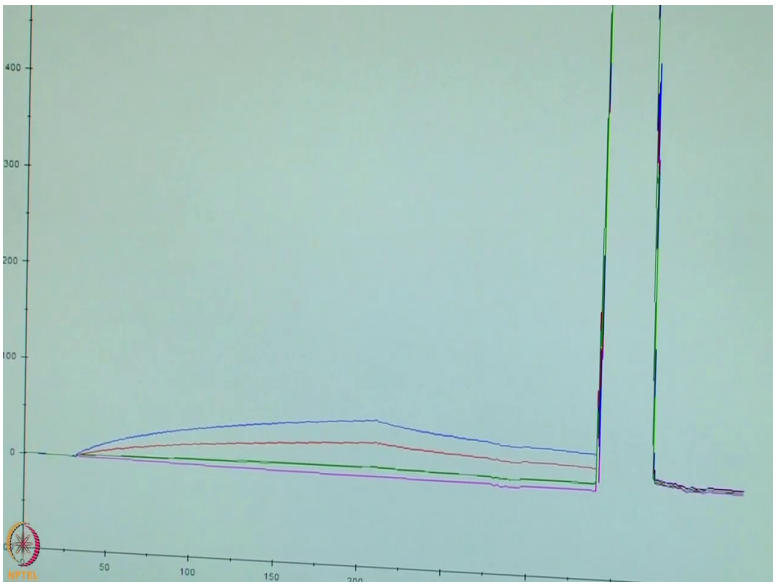
Now, our data is baseline to 0. If we want we can as well go on tools, colour, sample.

(Refer Slide Time: 22:12)



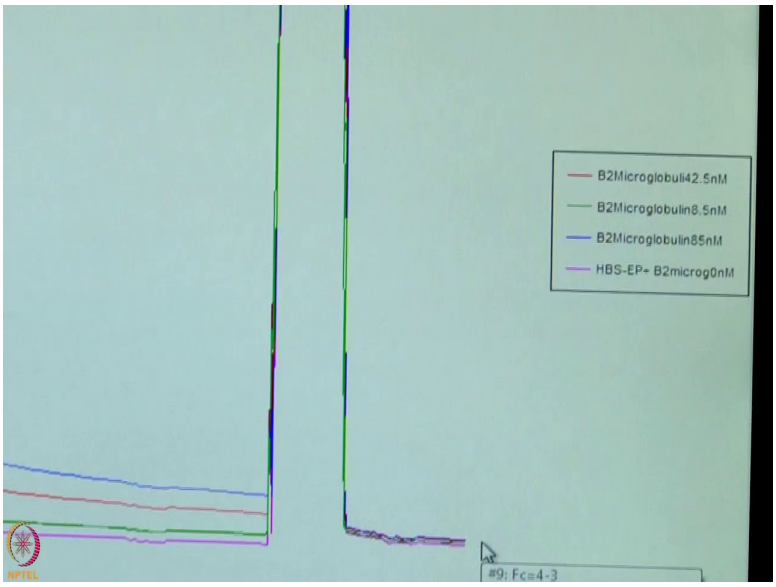
And we will see the different samples in different colours with the ligands on one side of the screen.

(Refer Slide Time: 22:18)



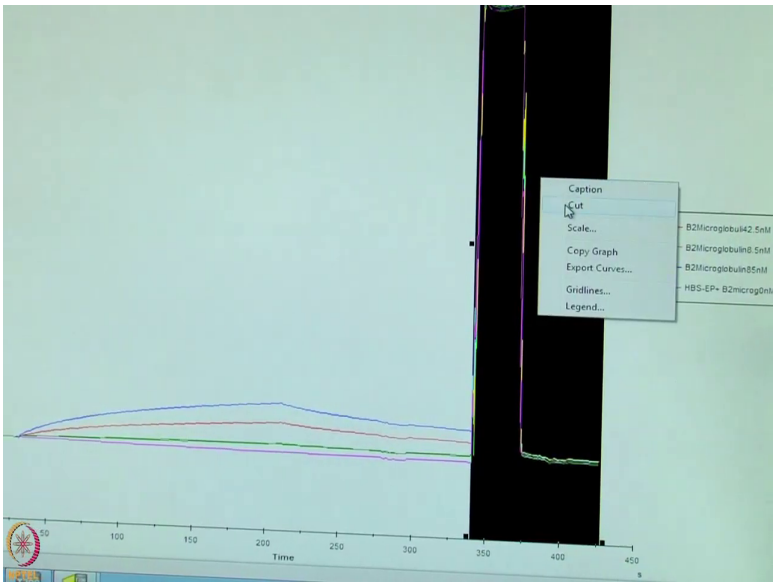
Here we have low, medium and high concentrations of beta 2 microglobulin injected over anti-beta 2M.

(Refer Slide Time: 22:29)



We could subtract or delete the regeneration area. Cut.

(Refer Slide Time: 22:46)



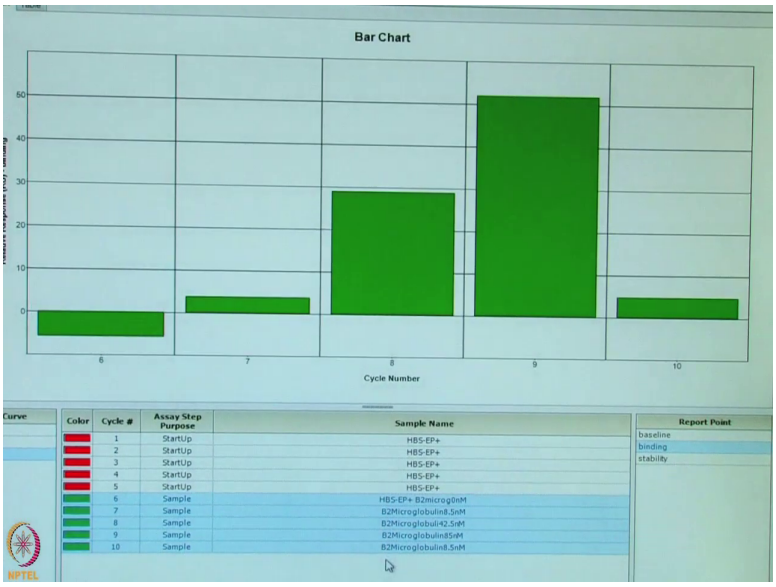
(Refer Slide Time: 22:58)

And now we can see our different concentrations of beta 2M with one of them in duplicate. So, these definitely binding of the beta 2M to anti-beta 2M antibody in a dose dependent manner.

(Refer Slide Time: 23:35)

The data can also been shown in form of a bar chart with all our red startup runs and green as our sample runs.

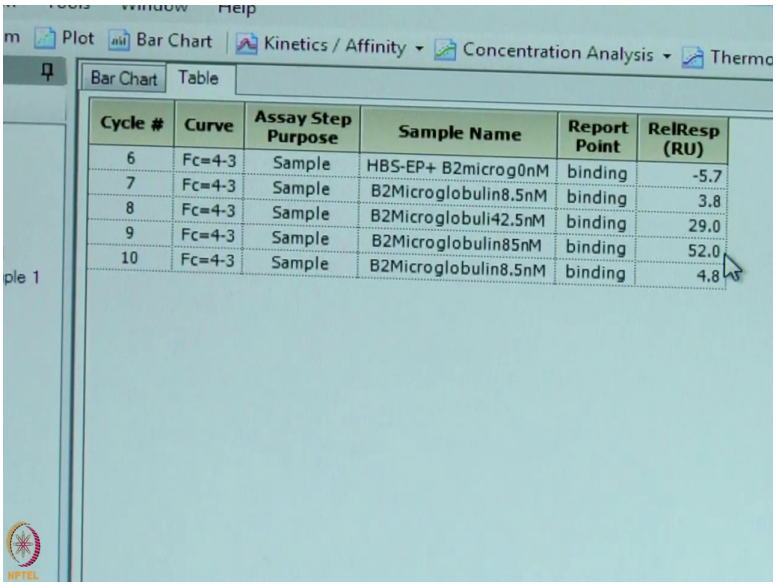
(Refer Slide Time: 24:02)



Here we will highlight the sample runs only and see cycle 7, 8 and 9 are our low, medium and high concentrations data which are dose dependent binding

Going to an other tab. We can see the relative responses of each molecule from the binding at 4 for the duplicate and medium at 29 and high at 52.

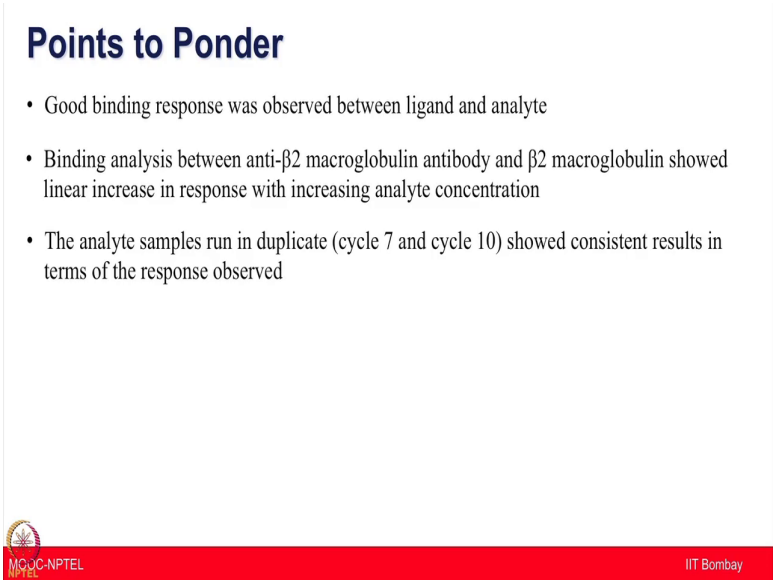
(Refer Slide Time: 24:39)



Cycle #	Curve	Assay Step Purpose	Sample Name	Report Point	RelResp (RU)
6	Fc=4-3	Sample	HBS-EP+ B2microg0nM	binding	-5.7
7	Fc=4-3	Sample	B2Microglobulin8.5nM	binding	3.8
8	Fc=4-3	Sample	B2Microglobuli42.5nM	binding	29.0
9	Fc=4-3	Sample	B2Microglobulin85nM	binding	52.0
10	Fc=4-3	Sample	B2Microglobulin8.5nM	binding	4.8

With this we will conclude our binding session and we prepare now for a kinetic experiment.

(Refer Slide Time: 25:23)



I hope you were able to follow the lab demonstration session. These protein interactions are identified using wide array of applications, however what is also required is an understanding of the extent to which these interactions actually occurs. Therefore, performing protein-protein interaction studies and calculating their kinetic values becomes very crucial.

We will continue the lab demonstrations and our discussion on SPR experiments for the kinetics analysis in the next lecture.

Thank you.