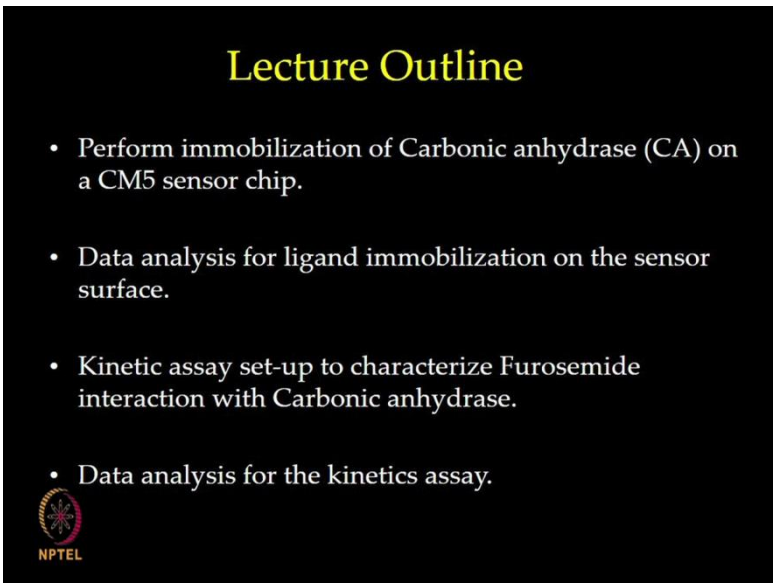
Interactomics: Protein Arrays and Label-Free Biosensors.
Professor Sanjeeva Srivastava.
Department of Biosciences and Bioengineering.
Indian Institute of Technology, Bombay.
Lecture-11.
Proetein-Small Molecule Interaction Study: Kinetic Analysis.

Welcome to MOOC NPTEL Course on Interatomics. As discussed in the last lecture, Surface Plasmon Resonance is increasingly being used inter discovery and validation studies using small molecules. In our previous lecture the binding analysis of small molecules to carbonic anhydrase, and human serum albumin was demonstrated and screening results were evaluated. The assay performance was studied and binding responses at active and reference surfaces were examined.

Today SPR biosensors offer exceptional sensitivity, and high quality kinetics covering a broad range of off and on rates, enabling most challenging assay of analyzing low molecular weight compounds with target molecules.

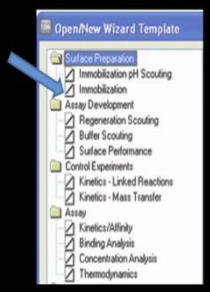
The ability to work with samples containing organic solvents DMSO is a challenging task which is now supported by these SPR Biosensors. One of the eight drug molecules florsemide dissolved in DMSO, from the previous screening assay will be further characterized for rapid kinetic analysis with carbonic anhydrase as immobilized targets using standardized compound concentration series, and DMSO solvent correction curve.

(Refer Slide Time: 2:26)

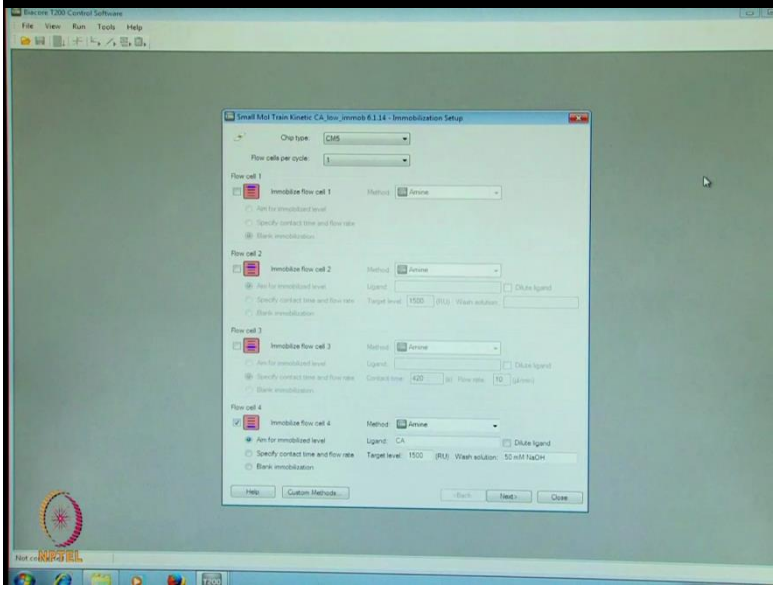


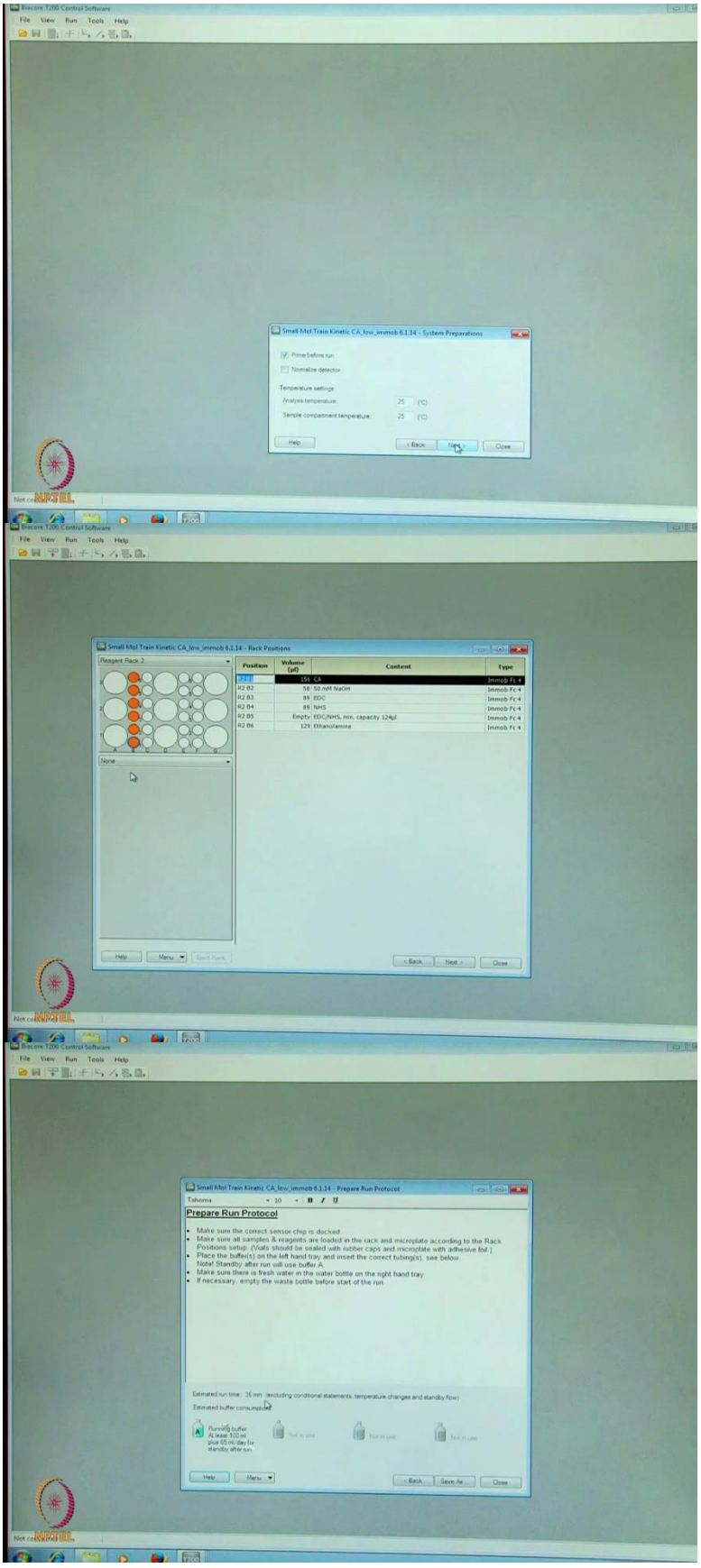
Immobilization of ligand and Kinetic assay set-up

Immobilization assay set-up



Low immobilization of carbonic anhydrase (CA) for kinetic analysis of the interaction between CA with furosemide.

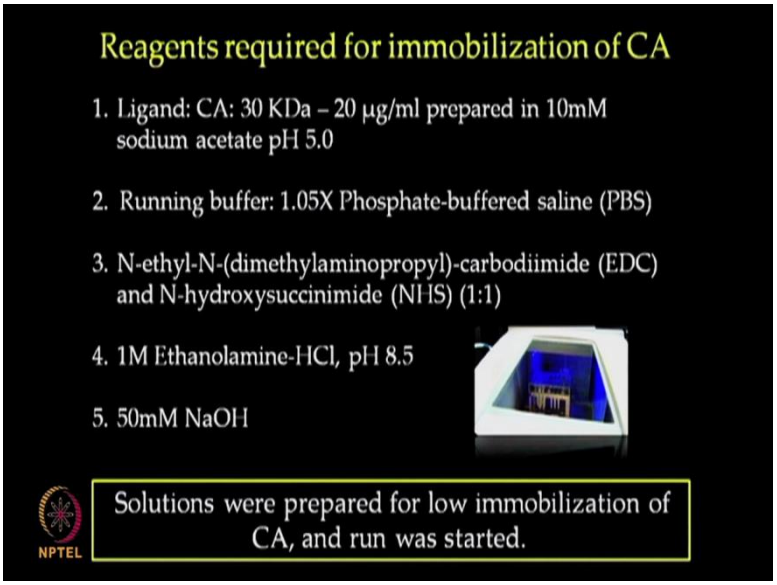



Biacore T200 Control Software
File View Run Tools Help
Small Mol Train Kinetic CA_low_immob 6.1.14 - System Preparations
Prime before run
Normalize detector
Temperature settings
Analysis temperature: 25 (°C)
Sample compartment temperature: 25 (°C)
Help
< Back
Next >
Close
Small Mol Train Kinetic CA_low_immob 6.1.14 - Rack Positions
Reagent Rack 2
None
Position
Volume (µl)
Content
Type
R2 B1 155 CA Immob Fc 4
R2 B2 58 50 mM NaOH Immob Fc 4
R2 B3 89 EDC Immob Fc 4
R2 B4 89 NHS Immob Fc 4
R2 B5 Empty EDC/NHS, min. capacity 124µl Immob Fc 4
R2 B6 129 Ethanolamine Immob Fc 4
Help
Menu
Eject Rack
< Back
Next >
Close
Small Mol Train Kinetic CA_low_immob 6.1.14 - Prepare Run Protocol
Tahoma 10
Prepare Run Protocol
Make sure the correct sensor chip is docked
Make sure all samples & reagents are loaded in the rack and microplate according to the Rack Positions setup. (Vials should be sealed with rubber caps and microplate with adhesive foil.)
Place the buffer(s) on the left hand tray and insert the correct tubing(s), see below.
Note! Standby after run will use buffer A.
Make sure there is fresh water in the water bottle on the right hand tray.
If necessary, empty the waste bottle before start of the run.
Estimated run time: 26 min (excluding conditional statements, temperature changes and standby flow)
Estimated buffer consumption:
A
Running buffer
At least 100 ml
plus 85 ml/day for standby after run.
Not in use
Not in use
Not in use
Help
Menu
< Back
Save As...
Close

We will now proceed with the immobilization and kinetic characterization of small molecule with target protein. Let us have the lab experimental session now. Now we will set up our immobilization for kinetic template. For this kinetic experiment we will have carbonic anhydrase immobilized by amine coupling on a flow channel 4. So the target level of 1500 RUs, and we leave immobilization as 0 on flow cell 3.

We go to the next tab, prime before run is highlighted here, analysis temperature at 25. Now we will have following reagents prepared for the kinetic analysis of interaction of carbon anhydrase with various molecules. Once the template is prepared, we go on to the next tab and we identify the run time of 36 minutes with a buffer consumption of at least 100ml for this particular session.

(Refer Slide Time: 5:28)

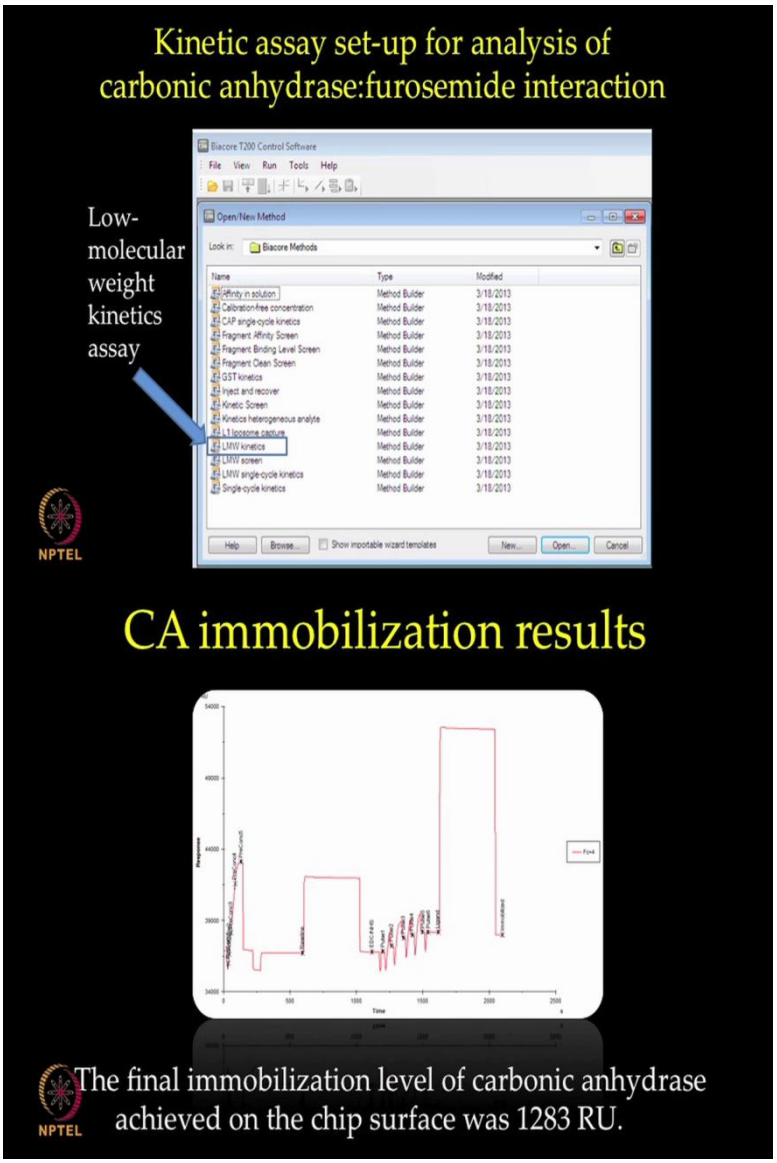


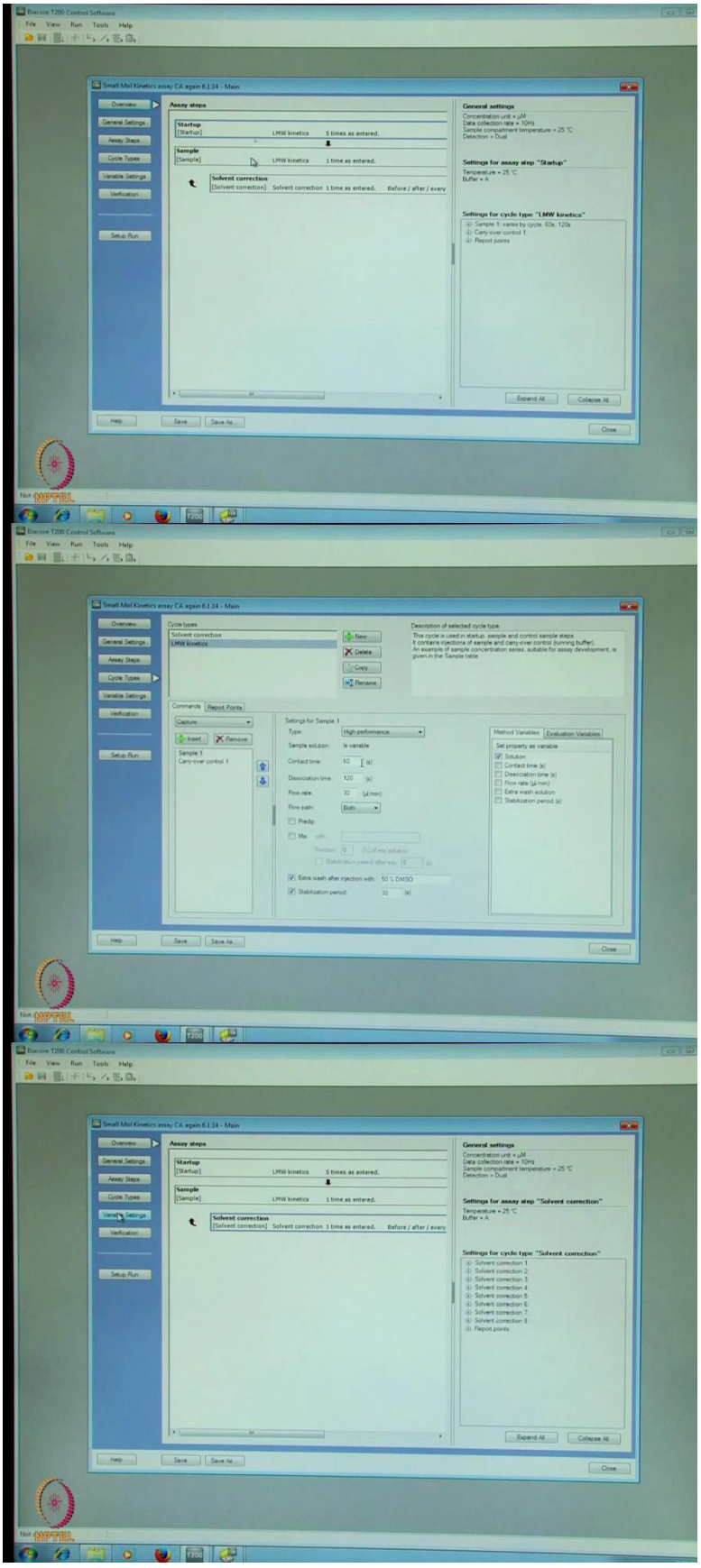
As per the volume listed on the table we will prepare buffer reagents required for immobilization of Carbonic Anhydrase on the sensor chip. Ligand is prepared at a working concentration of 20 microgram per ml using an immobilization buffer of 10 Millimolar of sodium acetate pH 5. We will be using 1.05 X PBS buffer at the running buffer which will be connected to the system followed by priming. EDC and NHS are prepared as suggested in the main coupling kit for surface activation. 1 molar Ethanolamine HCL pH 8 point 5 is prepared for blocking free extra groups on the surface. Lastly, 50 Millimolar NaOH was prepared which will be used electrostatically bound ligand.

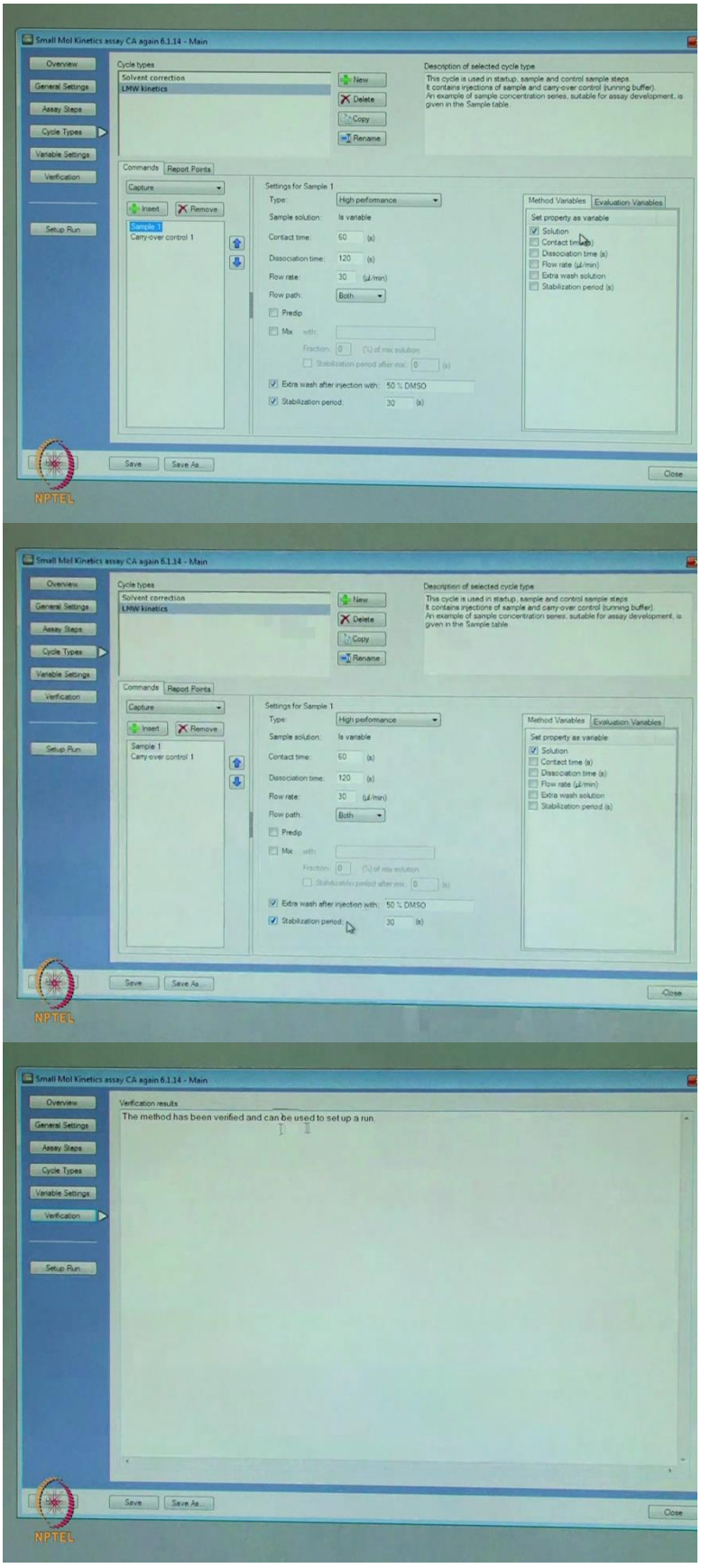
After preparations of these samples and reagents we transfer the sample tubes to the appropriate rack and start the run as shown for Beta 2 Microglobulin immobilization in

the earlier lectures. We will hence look at the immobilization result obtained from this run. Since it is recommended to use lower immobilization level for kinetic assay compared to screening assays, we targeted an immobilization level of 1500RU for carbonic anhydrase.

(Refer Slide Time: 6:10)

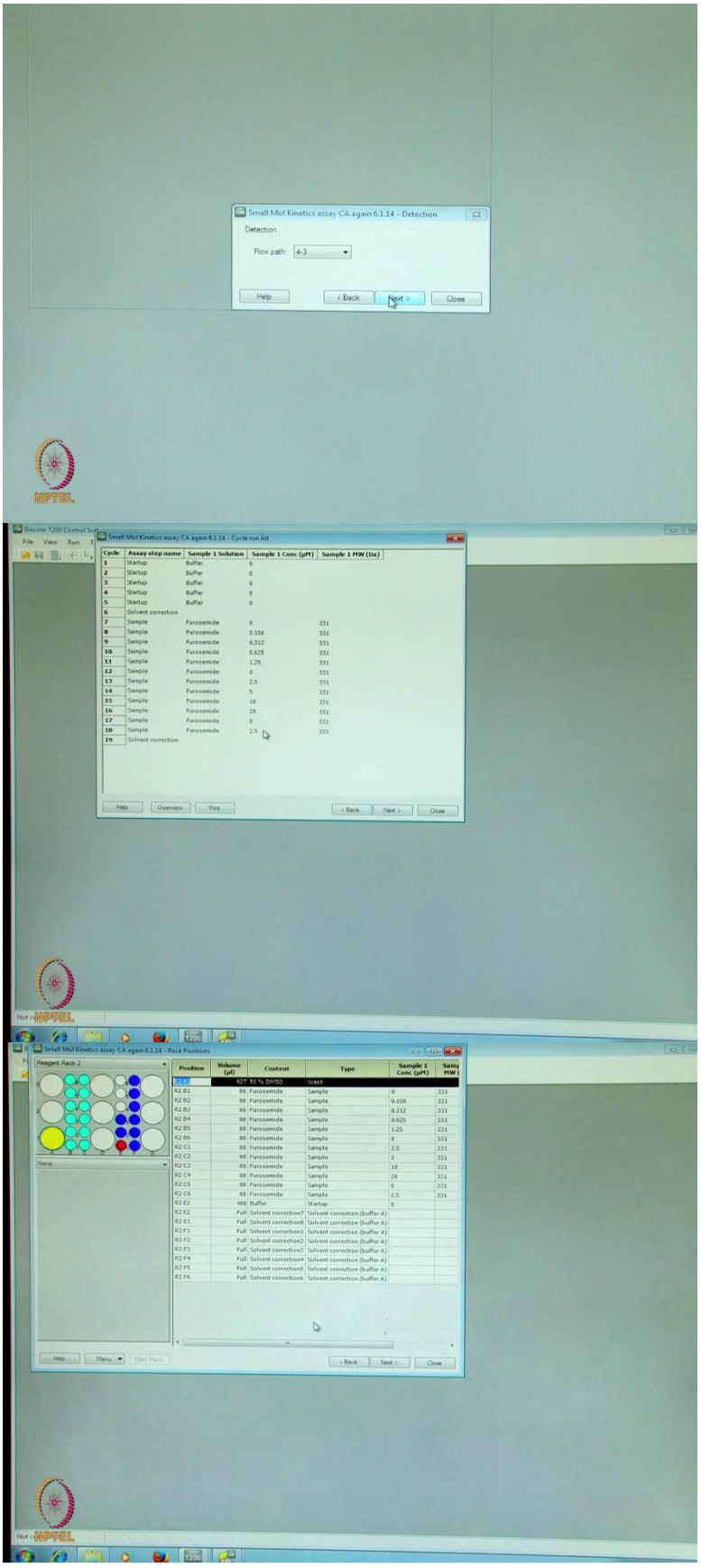



Biacore T200 Control Software
File View Run Tools Help
Small Mol Kinetics assay CA again 8.1.14 - Main
Overview
General Settings
Assay Steps
Cycle Types
Variable Settings
Verification
Setup Run
Assay steps
Startup
[Startup]
LMW kinetics
5 times as entered.
Sample
[Sample]
LMW kinetics
1 time as entered.
Solvent correction
[Solvent correction]
Solvent correction
1 time as entered.
Before / after / every
General settings
Concentration unit = µM
Data collection rate = 10Hz
Sample compartment temperature = 25 °C
Detection = Dual
Settings for assay step "Startup"
Temperature = 25 °C
Buffer = A
Settings for cycle type "LMW kinetics"
Sample 1: varies by cycle, 60s, 120s
Carry-over control 1
Report points
Expand All
Collapse All
Help
Save
Save As...
Close
Biacore T200 Control Software
File View Run Tools Help
Small Mol Kinetics assay CA again 8.1.14 - Main
Overview
General Settings
Assay Steps
Cycle Types
Variable Settings
Verification
Setup Run
Cycle types
Solvent correction
LMW kinetics
New
Delete
Copy
Rename
Description of selected cycle type
This cycle is used in startup, sample and control sample steps
It contains injections of sample and carry-over control (running buffer).
An example of sample concentration series, suitable for assay development, is given in the Sample table.
Commands
Report Points
Capture
Insert
Remove
Sample 1
Carry-over control 1
Settings for Sample 1
Type:
High performance
Sample solution:
Is variable
Contact time:
60
(s)
Dissociation time:
120
(s)
Flow rate:
30
(µl/min)
Flow path:
Both
Predip
Mix
with:
Fraction:
0
(%) of mix solution
Stabilization period after mix:
0
(s)
Extra wash after injection with:
50 % DMSO
Stabilization period:
30
(s)
Method Variables
Evaluation Variables
Set property as variable
Solution
Contact time (s)
Dissociation time (s)
Flow rate (µl/min)
Extra wash solution
Stabilization period (s)
Help
Save
Save As...
Close
Biacore T200 Control Software
File View Run Tools Help
Small Mol Kinetics assay CA again 8.1.14 - Main
Overview
General Settings
Assay Steps
Cycle Types
Variable Settings
Verification
Setup Run
Assay steps
Startup
[Startup]
LMW kinetics
5 times as entered.
Sample
[Sample]
LMW kinetics
1 time as entered.
Solvent correction
[Solvent correction]
Solvent correction
1 time as entered.
Before / after / every
General settings
Concentration unit = µM
Data collection rate = 10Hz
Sample compartment temperature = 25 °C
Detection = Dual
Settings for assay step "Solvent correction"
Temperature = 25 °C
Buffer = A
Settings for cycle type "Solvent correction"
Solvent correction 1
Solvent correction 2
Solvent correction 3
Solvent correction 4
Solvent correction 5
Solvent correction 6
Solvent correction 7
Solvent correction 8
Report points
Expand All
Collapse All
Help
Save
Save As...
Close


Small Mol Kinetics assay CA again 6.1.14 - Main
Overview
General Settings
Assay Steps
Cycle Types
Variable Settings
Verification
Setup Run
Cycle types
Solvent correction
LMW kinetics
New
Delete
Copy
Rename
Description of selected cycle type
This cycle is used in startup, sample and control sample steps.
It contains injections of sample and carry-over control (running buffer).
An example of sample concentration series, suitable for assay development, is given in the Sample table.
Commands
Report Points
Capture
Insert
Remove
Sample 1
Carry-over control 1
Settings for Sample 1
Type:
High performance
Sample solution:
Is variable
Contact time:
60
(s)
Dissociation time:
120
(s)
Flow rate:
30
(µl/min)
Flow path:
Both
Predip
Mix
with:
Fraction:
0
(%) of mix solution
Stabilization period after mix:
0
(s)
Extra wash after injection with:
50 % DMSO
Stabilization period:
30
(s)
Method Variables
Evaluation Variables
Set property as variable
Solution
Contact time (s)
Dissociation time (s)
Flow rate (µl/min)
Extra wash solution
Stabilization period (s)
Save
Save As...
Close
NPTEL

Small Mol Kinetics assay CA again 6.1.14 - Main
Overview
General Settings
Assay Steps
Cycle Types
Variable Settings
Verification
Setup Run
Cycle types
Solvent correction
LMW kinetics
New
Delete
Copy
Rename
Description of selected cycle type
This cycle is used in startup, sample and control sample steps.
It contains injections of sample and carry-over control (running buffer).
An example of sample concentration series, suitable for assay development, is given in the Sample table.
Commands
Report Points
Capture
Insert
Remove
Sample 1
Carry-over control 1
Settings for Sample 1
Type:
High performance
Sample solution:
Is variable
Contact time:
60
(s)
Dissociation time:
120
(s)
Flow rate:
30
(µl/min)
Flow path:
Both
Predip
Mix
with:
Fraction:
0
(%) of mix solution
Stabilization period after mix:
0
(s)
Extra wash after injection with:
50 % DMSO
Stabilization period:
30
(s)
Method Variables
Evaluation Variables
Set property as variable
Solution
Contact time (s)
Dissociation time (s)
Flow rate (µl/min)
Extra wash solution
Stabilization period (s)
Save
Save As...
Close
NPTEL

Small Mol Kinetics assay CA again 6.1.14 - Main
Overview
General Settings
Assay Steps
Cycle Types
Variable Settings
Verification
Setup Run
Verification results
The method has been verified and can be used to set up a run.
Save
Save As...
Close
NPTEL



As shown in the figure the immobilization procedure resulted in final immobilization level of 1283 RU. With this we proceed further with setting up of kinetic assay result for low

molecule weight compound to study the interaction between carbonic anhydrase ,and furosemide.

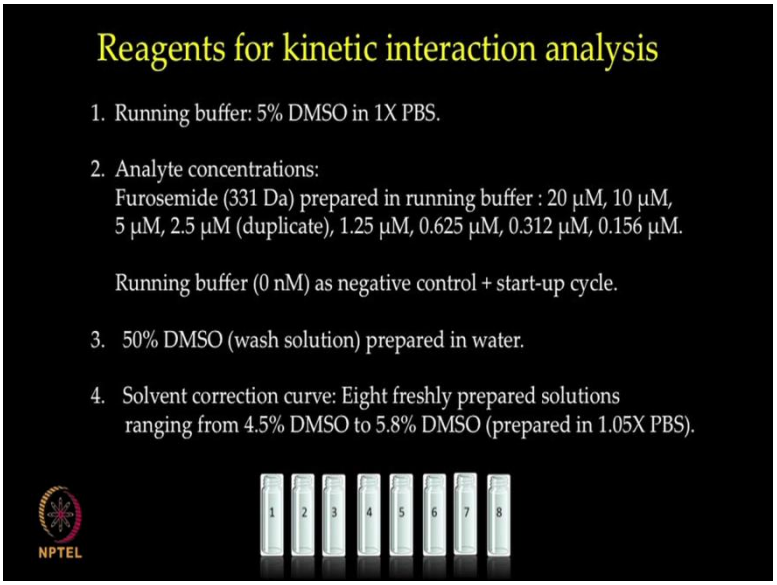
We now see how a small molecule kinetic template is set up. Similarly here as well we have the start up, we have a sample flown at least one on the surface, and a solvent correction with 8 different solvent correction points set here. Here also in the variable setting or in the cycle type we go to the low molecular weight kinetic result, check that the type is high performance.

Contact time is 60 seconds, dissociation time is 120 seconds, fluoride at 30 Microliter per minute, flow path both, extra wash with 50 percent DMSO, stabilization time of 30 seconds is provided here. Also there is a carry over control injection will be given at a flow rate of 40 Microliter per minute. Similarly the sample solution is available here, also the concentration and the molecular weight are variable here.

So we go into the verification mode, and see that the method have been verified and can be used through set up a run. We go into the set up, here the flow path is 4 minus 3. We go on next, we have 5 start up cycles, one set of solvent correction before the run, and another solvent correction after the run. In between we have furosemide injected at various concentrations.

On the surface of the Carbonic Anhydrase, and the kinetic data is accumulated here. We have highlighted prime before run and following are the track positions for various samples with furosemide at different concentration. 50 percent DMSO buffer for the startup solvent correction, 8 different solvent correction sample vials provided for a successful kinetic experimentation of furosemide binding to carbonic anhydrase.

(Refer Slide Time: 10:52)



As per the volumes listed in the table we prepare the following samples and reagents. We prepare a running buffer in 5 percent of DMSO in 1X PBS. We will be preparing a total of 5 concentrations of furosemide starting from 20 micromolar, 10 micromolar, 5 micromolar, 2 point 5 micromolar, 1 point 25 micromolar, point 625 micromolar, point 312 micromolar, and point 156 micromolar using a twofold serial dilution solution in the running buffer with 2 point 5 micromolar concentration in duplicate.

Running buffer will be used as 0 concentration negative control as well as startup cycles. A wash solution is prepared with 50 percent DMSO in water. Lastly, 8 freshly prepared DMSO solutions ranging from 4 point 5 percent to 5 point 8 percent DMSO are prepared in 1 point 05X PBS for solvent correction.

(Refer Slide Time: 11:04)

We will now work on the reagent required for a kinetic assay to study interaction between human serum albumin and furosemide. In this study we will again be using 5 percent DMSO in 1 point 05X PBS as the running buffer which will also be used for the initial start up cycle. As prepared in the screening assay, we will again prepare 8 different solvent solution ranging from 4 point 5 percent DMSO in PBS to 5 point 8 percent DMSO in PBS by mixing these two solutions in different ratios.

To reiterate we have numbered the tubes as 1 to 8 and added 200, 400, 600, 800, 1000, 1200, and 1400 microliter of 4 point 5 percent of DMSO in tubes 2 to 8 respectively. Following this we added 1400, 1200, 1000, 800, 600, 400, and 200 microliters of 5 point 8 percent DMSO in tubes 1 to 7 respectively.

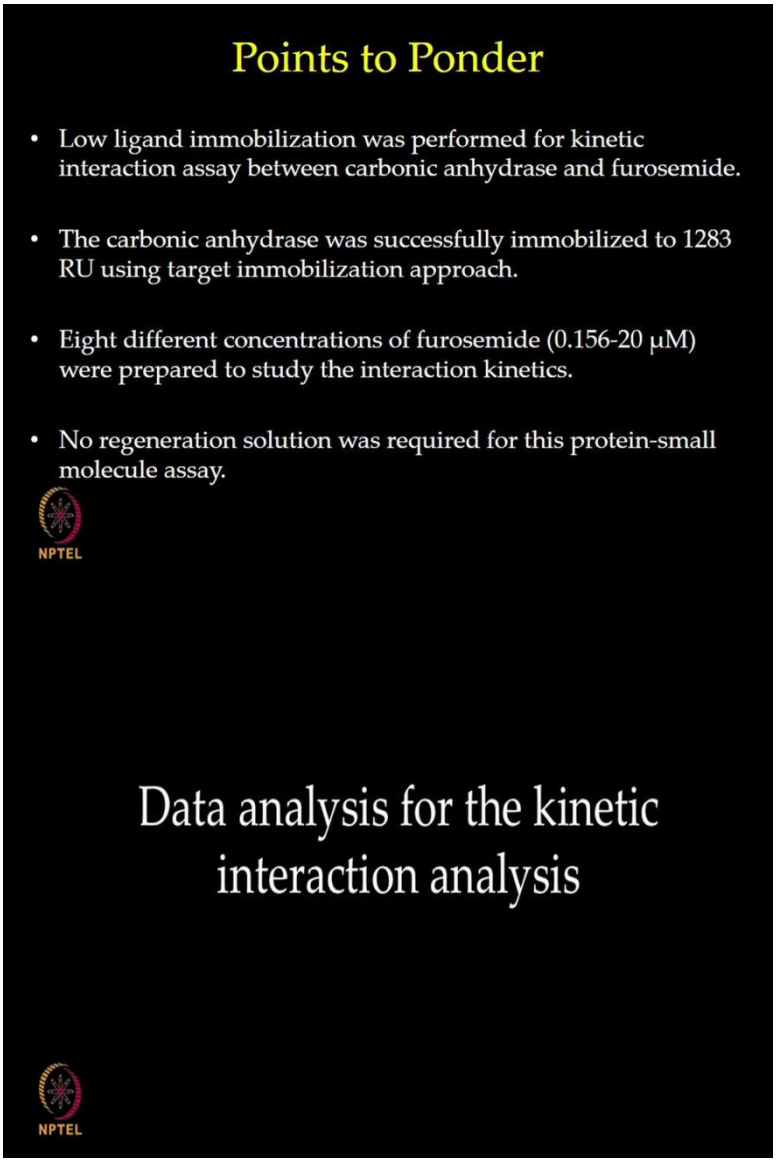
We will mix these solutions vigorously and put the lids on and keep them in dark till we are ready all the other samples. We will be using 8 different concentrations of analyte furosemide in the running buffer including 20 Micromolar, 10 micromolar, 5 Micromolar, 2 point 5 micromolar, 1 point 25 micromolar, point 625 micromolar, point 312 micromolar, and point 156 micromolar. We will prepare this concentration series in two fold dilution using running buffer starting from 20 micromolar, which means within 200 microliter of 20 micromolar protein with 200 microliter of running buffer to get 10 micromolar concentration.

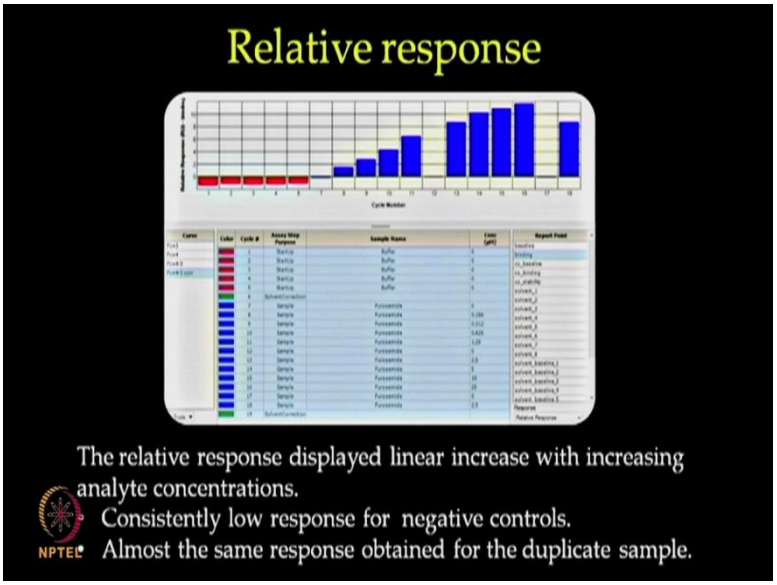
We similarly continue the two fold dilution into rest of the concentration series. One of the concentrations should be running duplicate. So here in this experiment we will be running 2

point 5 micromolar concentration in duplicate, and we will also include 3 zero concentration which will be nothing but running buffer. All of these samples and reagents have now been allicoted in the required volumes in these specialized tubes.

We will now proceed to insert these tubes including the samples, the startup, 50 percent DMSO, and the solvent correction solution into the appropriate rack and then into the system to perform the protein small molecules screening assay. We have now placed all the tubes inside the rack including wash solution, the furosemide samples, the startup, and solvent correction solution and now we will place this rack inside the system to start with our kinetic assay. We will not start with our run as demonstrated in our earlier lectures.

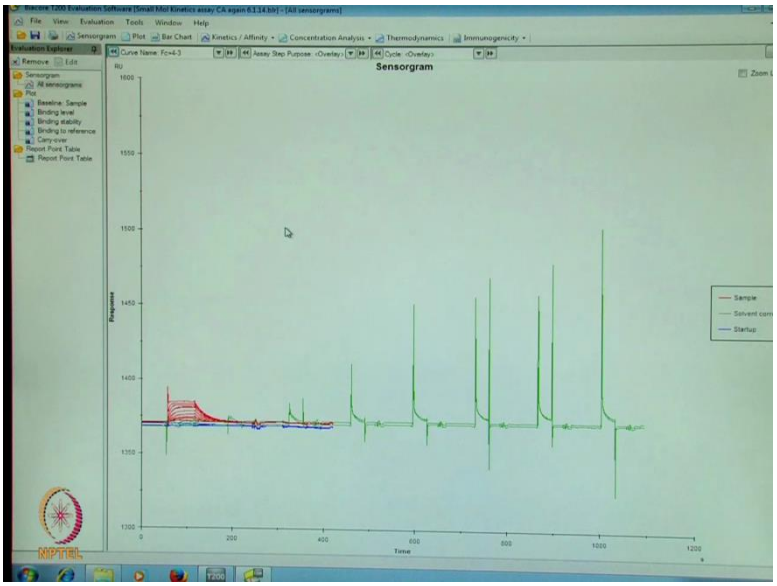
(Refer Slide Time: 15:25)

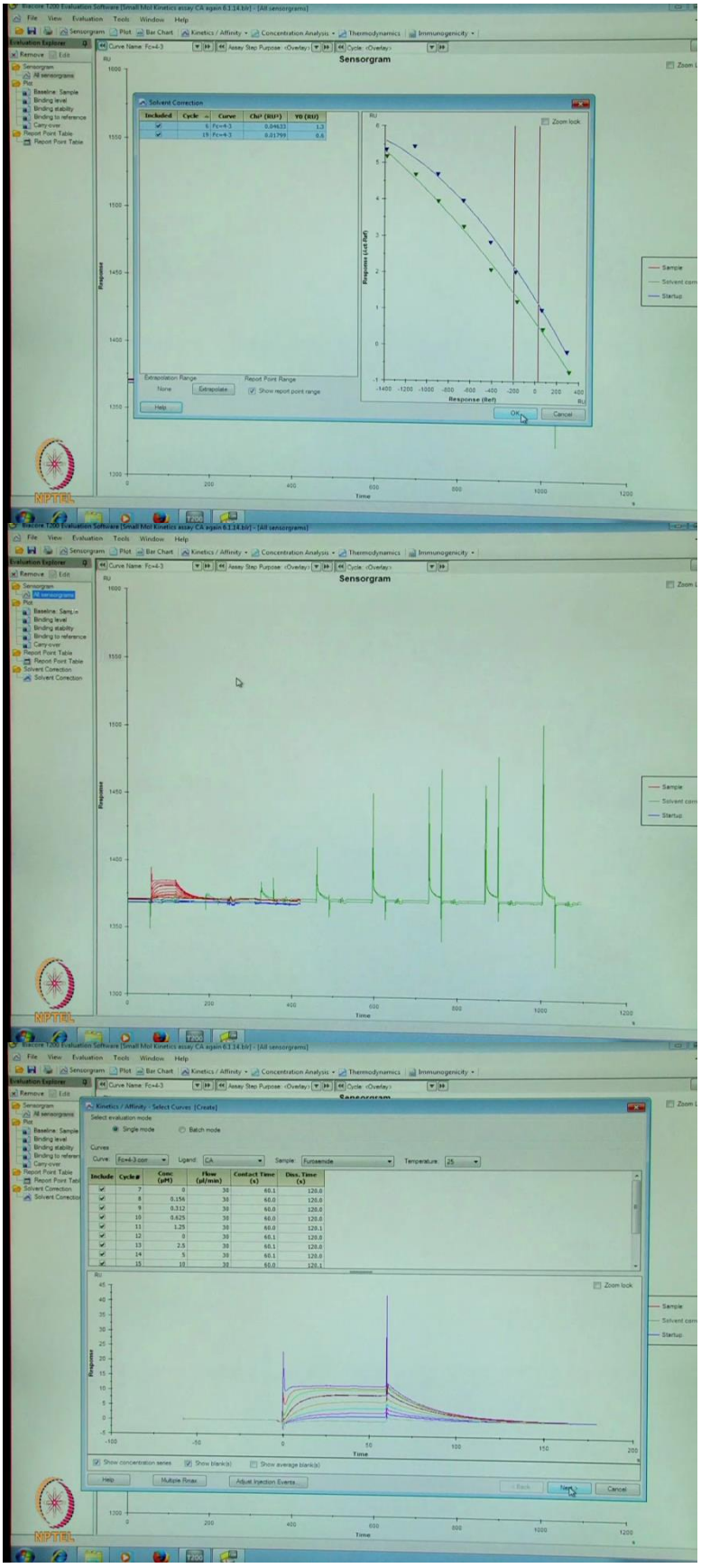


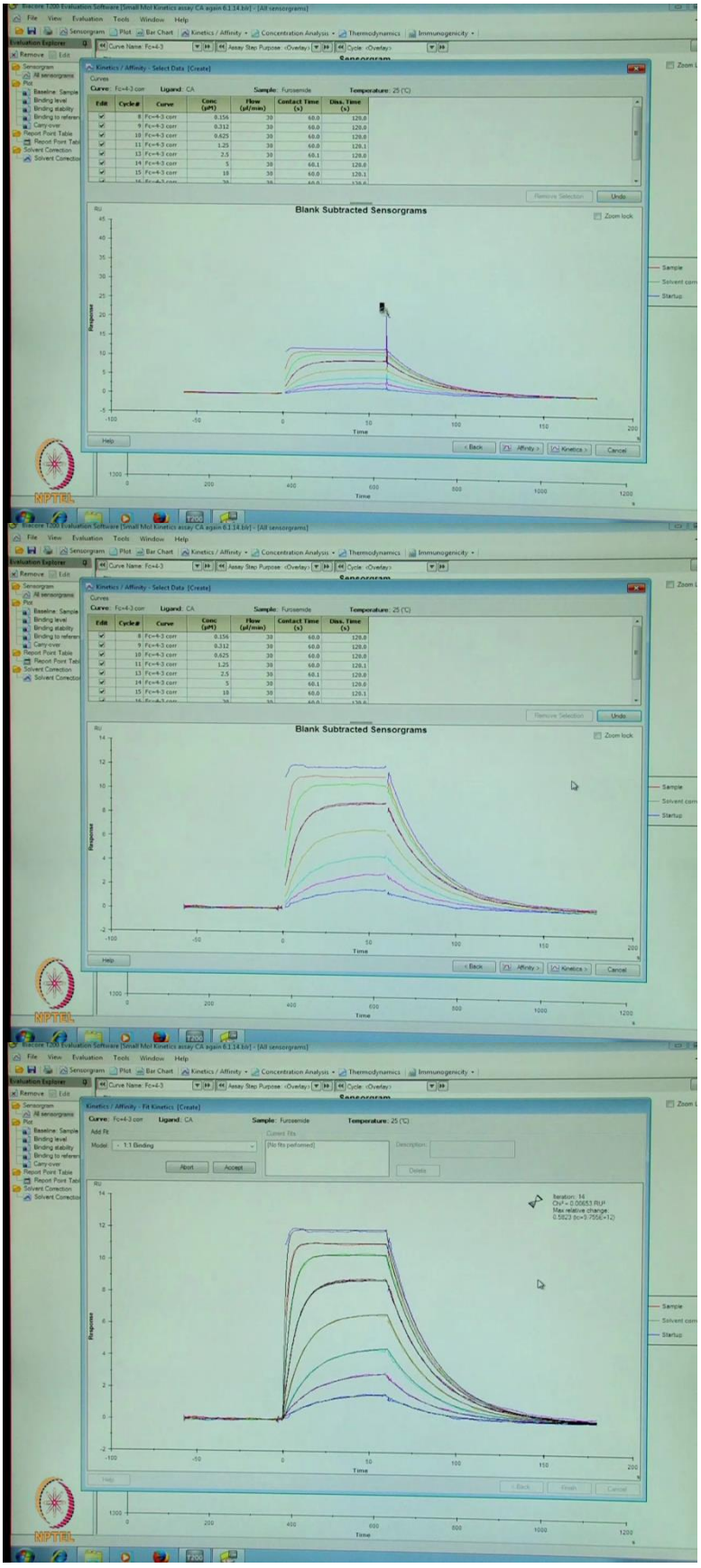


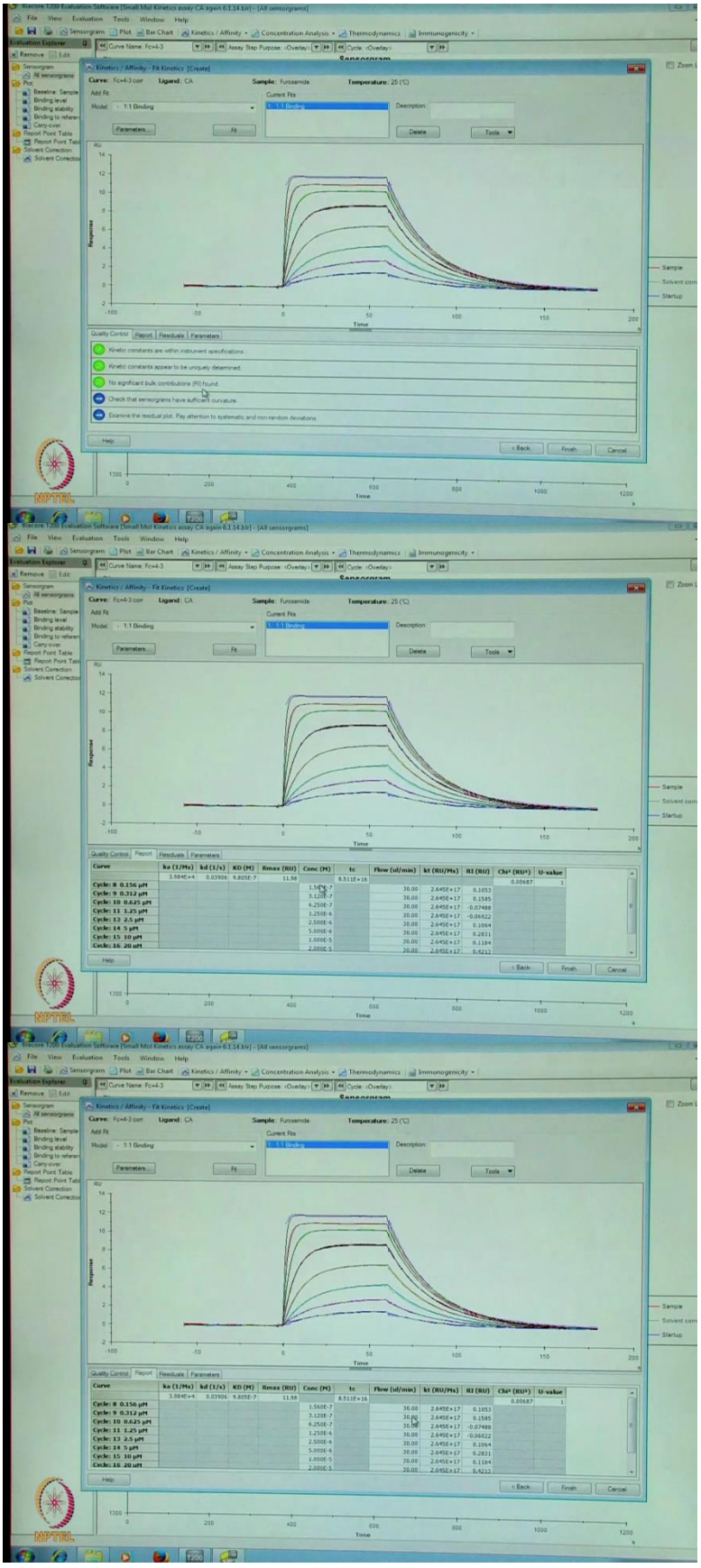
Before starting with the data analysis on kinetics of interactions between carbonic anhydrase and furosemide, we would like to show the diagram obtained from the Sensorgram which represents the relative response for report point binding again cycle numbers for samples. As reflected in the graph, the relative response displayed linear increase with increasing analyte concentrations with consistently low response for negative controls shown by cycle 7, 12, and 17, and almost the same response obtained for the duplicate sample that is 2 point 5 micromolar shown by cycle 13 and 18.

(Refer Slide Time: 16:38)




Sensorgram
Solvent Correction
Included
Cycle
Curve
Chi² (RU²)
Y0 (RU)
Zoom lock
Response (Ref)
Extrapolation Range
Report Point Range
None
Extrapolate
Show report point range
Help
OK
Cancel
Sample
Startup
Response
Time
Sensorgram
Sample
Startup
Response
Time
Kinetics / Affinity : Select Curves [Create]
Select evaluation mode
Single mode
Batch mode
Curves
Include
Cycle#
Conc (pM)
Flow (µl/min)
Contact Time (s)
Diss. Time (s)
Zoom lock
Show concentration series
Show blank(s)
Show average blank(s)
Help
Multiple Rmax
Adjust Injection Events
Back
Next
Cancel
Sample
Startup
Response
Time


Biacore T200 Evaluation Software [Small Mol Kinetics assay CA again 6.1.14.blk] - [All sensorgrams]
File View Evaluation Tools Window Help
Sensorgram Plot Bar Chart Kinetics / Affinity Concentration Analysis Thermodynamics Immunogenicity
Kinetics / Affinity - Select Data [Create]
Curves
Curve: Fc+4-3 corr Ligand: CA Sample: Furosemide Temperature: 25 (°C)
Edit Cycle# Curve Conc (µM) Flow (µl/min) Contact Time (s) Diss. Time (s)
8 Fc+4-3 corr 0.156 30 60.0 120.0
9 Fc+4-3 corr 0.312 30 60.0 120.0
10 Fc+4-3 corr 0.625 30 60.0 120.0
11 Fc+4-3 corr 1.25 30 60.0 120.1
13 Fc+4-3 corr 2.5 30 60.1 120.0
14 Fc+4-3 corr 5 30 60.1 120.0
15 Fc+4-3 corr 10 30 60.0 120.1
Remove Selection Undo
Blank Subtracted Sensorgrams
Zoom lock
Ru
Response
Time
Help
< Back Affinity > Kinetics > Cancel
Biacore T200 Evaluation Software [Small Mol Kinetics assay CA again 6.1.14.blk] - [All sensorgrams]
File View Evaluation Tools Window Help
Sensorgram Plot Bar Chart Kinetics / Affinity Concentration Analysis Thermodynamics Immunogenicity
Kinetics / Affinity - Select Data [Create]
Curves
Curve: Fc+4-3 corr Ligand: CA Sample: Furosemide Temperature: 25 (°C)
Edit Cycle# Curve Conc (µM) Flow (µl/min) Contact Time (s) Diss. Time (s)
8 Fc+4-3 corr 0.156 30 60.0 120.0
9 Fc+4-3 corr 0.312 30 60.0 120.0
10 Fc+4-3 corr 0.625 30 60.0 120.0
11 Fc+4-3 corr 1.25 30 60.0 120.1
13 Fc+4-3 corr 2.5 30 60.1 120.0
14 Fc+4-3 corr 5 30 60.1 120.0
15 Fc+4-3 corr 10 30 60.0 120.1
Remove Selection Undo
Blank Subtracted Sensorgrams
Zoom lock
Ru
Response
Time
Help
< Back Affinity > Kinetics > Cancel
Biacore T200 Evaluation Software [Small Mol Kinetics assay CA again 6.1.14.blk] - [All sensorgrams]
File View Evaluation Tools Window Help
Sensorgram Plot Bar Chart Kinetics / Affinity Concentration Analysis Thermodynamics Immunogenicity
Kinetics / Affinity - Fit Kinetics [Create]
Curve: Fc+4-3 corr Ligand: CA Sample: Furosemide Temperature: 25 (°C)
Add Fit
Model: 1:1 Binding
Current Fits
[No fits performed]
Description:
Abort Accept Delete
Iteration: 14
Chi² = 0.00651 RU
Max relative change:
0.5823 (tc=3.7556[+1])
Ru
Response
Time
Help
< Back Finish Cancel


Biacore T200 Evaluation Software [Small Mol Kinetics assay CA again 6.1.14.blk] - [All sensorgrams]
File View Evaluation Tools Window Help
Sensorgram Plot Bar Chart Kinetics / Affinity Concentration Analysis Thermodynamics Immunogenicity
Evaluation Explorer
Kinetics / Affinity - Fit Kinetics [Create]
Curve: Fc4-3 corr Ligand: CA Sample: Furosemide Temperature: 25 (°C)
Add Fit
Model: 1:1 Binding
Current Fits
1:1 Binding
Description:
Parameters... Fit Delete Tools
Sensorgram
RU
Response
Time
Quality Control Report Residuals Parameters
Kinetic constants are within instrument specifications.
Kinetic constants appear to be uniquely determined.
No significant bulk contributions (RI) found.
Check that sensorgrams have sufficient curvature.
Examine the residual plot. Pay attention to systematic and non-random deviations.
Help
< Back Finish Cancel
Sample
Solvent corr
Startup
NPTEL

Biacore T200 Evaluation Software [Small Mol Kinetics assay CA again 6.1.14.blk] - [All sensorgrams]
Kinetics / Affinity - Fit Kinetics [Create]
Curve: Fc4-3 corr Ligand: CA Sample: Furosemide Temperature: 25 (°C)
Model: 1:1 Binding
Parameters... Fit Delete Tools
Sensorgram
Quality Control Report Residuals Parameters
Curve ka (1/Ms) kd (1/s) KD (M) Rmax (RU) Conc (M) tc Flow (ul/min) kt (RU/Ms) RI (RU) Chi² (RU²) U-value
Cycle: 8 0.156 µM
Cycle: 9 0.312 µM
Cycle: 10 0.625 µM
Cycle: 11 1.25 µM
Cycle: 12 2.5 µM
Cycle: 14 5 µM
Cycle: 15 10 µM
Cycle: 16 20 µM
Help
< Back Finish Cancel
Sample
Solvent corr
Startup
NPTEL

Biacore T200 Evaluation Software [Small Mol Kinetics assay CA again 6.1.14.blk] - [All sensorgrams]
Kinetics / Affinity - Fit Kinetics [Create]
Curve: Fc4-3 corr Ligand: CA Sample: Furosemide Temperature: 25 (°C)
Model: 1:1 Binding
Parameters... Fit Delete Tools
Sensorgram
Quality Control Report Residuals Parameters
Curve ka (1/Ms) kd (1/s) KD (M) Rmax (RU) Conc (M) tc Flow (ul/min) kt (RU/Ms) RI (RU) Chi² (RU²) U-value
Cycle: 8 0.156 µM
Cycle: 9 0.312 µM
Cycle: 10 0.625 µM
Cycle: 11 1.25 µM
Cycle: 12 2.5 µM
Cycle: 14 5 µM
Cycle: 15 10 µM
Cycle: 16 20 µM
Help
< Back Finish Cancel
Sample
Solvent corr
Startup
NPTEL

Let us now have a more detailed data analysis on the kinetic assay. Now we will look at the kinetic data generated from the interaction of carbonic anhydrase with furosemide. The data looks like this way with a sample solvent correction at the startup.

Now to evaluate this data we have to go into the evaluation add solvent correction, and once solvent correction data looks okay, say okay, and the data is now subtracted from Sensorgram. From this again we go into the kinetic affinity mode, surface bound, data is shown here in different colors for Furosemide concentrations , we say next, and we clearly see the data having some spikes.

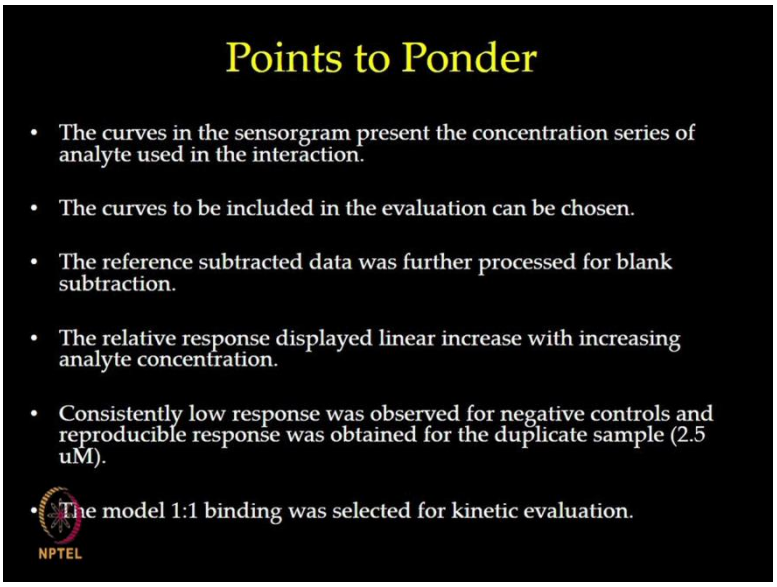
So using the mouse button we can highlight that range, subtract or remove the selected area, similarly we can do that here as well. Once the subtraction is done, we can go further to evaluate in the kinetic mode, and fit the data. So here on the quality control tab you see all of them green, and there is no significant bulk contribution found, and data is uniquely determined, and the data points are the kinetic on and off rates are within the range of the machines.

The report shows on rate, off rate, affinity constant, or binding constant, RMX of 1RU, various injected concentrations, flow rate, differences in the interactive index of the sample and the running buffer. Chi square below 1, and U value below 25. So this data shows the interaction of furosemide with carbonic anhydrase is 9 point 8 into 24 minus 7 molar.

With this we go to the next tab, and analyse the data. Here with the one-to-one fit of data the residuals or the differences from the black line to the colored line should fall within the green areas or at least within the red area. Here they are within the green areas, and they look absolutely good to proceed with this data.

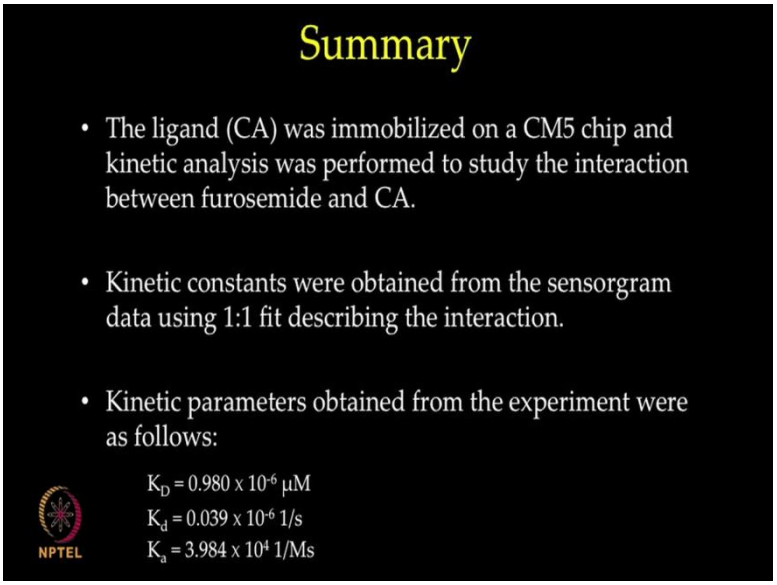
Similarly other parameter like the standard error is provided here. With this we will conclude the kinetic information generated for the interaction of furosemide with ligand carbonic anhydrase recorded at 25 degree centigrade, and solvent corrected for DMSO presence in the running and the sample buffers.

(Refer Slide Time: 20:48)



In last lectures we have discussed how to perform protein protein, and protein small molecule interaction studies for binding and kinetic analysis using Surface Plasmon Resonance label free platforms. Thank You.

(Refer Slide Time: 21:23)



References

- Huber et al. 2006. Biomolecular Interaction Analysis in Drug Discovery Using Surface Plasmon Resonance Technology. Current Pharmaceutical Design., 12, 3999-4021.
- Myszka and Rich 2000. Implementing surface plasmon resonance biosensors in drug discovery. Pharmaceutical Science & Technology Today., 3, 310-317.
- Nguyen, et al. 2009. Biosensor-surface plasmon resonance: Quantitative analysis of small molecule–nucleic acid interactions. Methods Mol Biol., 42, 150-161.
- Rich and Myszka 2004. Why you should be using more SPR biosensor technology. Drug Discovery Today: Technologies., 1, 301–308.
- Shankaran et al. 2007. Recent advancements in surface plasmon resonance immunosensors for detection of small molecules of biomedical, food and environmental interest. Sensors and Actuators B: Chemical., 121, 158-177.

Acknowledgment

Experimental demonstration and commentary by Dr. Veenita Shah (IIT Bombay) and Dr. Srinivas Sistla (GE Healthcare).