



USER MANUAL
SPRI DEMO CHIP KIT
SURFACE PLASMON RESONANCE IMAGING

GUIDE TO A DEMONSTRATION OF REAL SPRI-BASED ARRAY ASSAYS

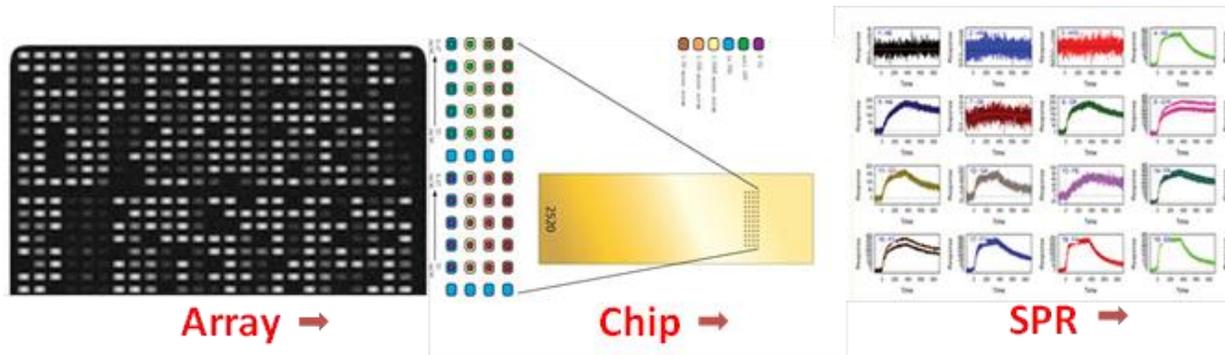
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Solutions for Functional Proteomics

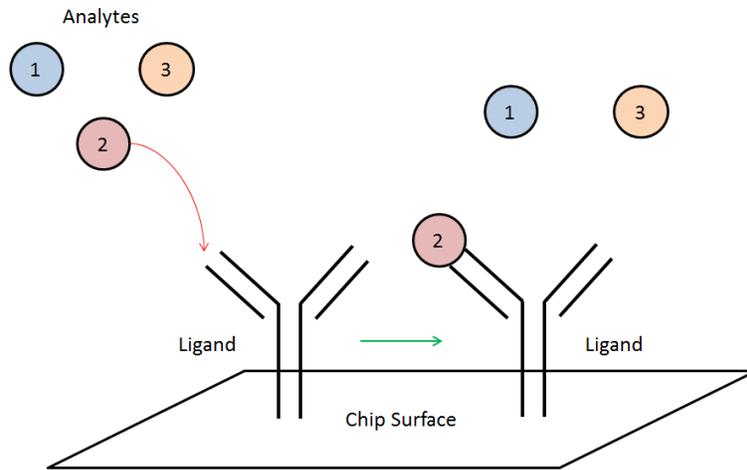
The screening, affinity, kinetics, and concentration of ligands and analytes are measured with the highest throughput and array density of the label-free SPRI system

Contents

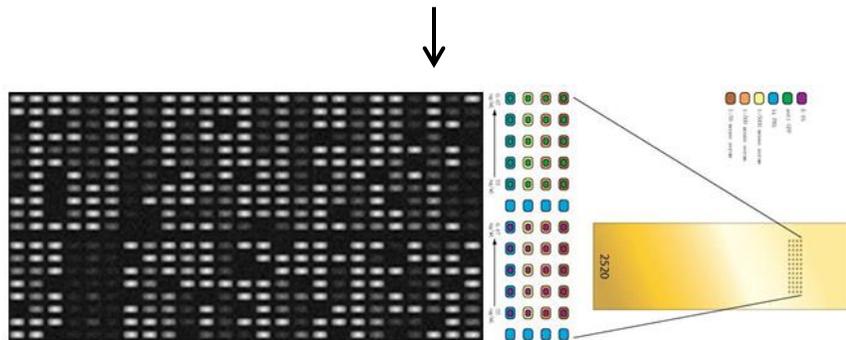
1. Introduction	2
2. Demo Chip Kit Description	3
3. Demo Chip Kit Components.....	3
Table 1 – List of Kit Components.....	4
4. Instructions	4
Table 2 – Demo Chip Setup	4
5. Procedures	5
Table 3 – Method and Procedure Guidelines.....	5
6. Typical Cases	5
6.1. Strong Binding	6
6.2. Weak Binding.....	7
6.3. Small-Large Molecule Interactions.....	7
6.4. Cross Reactivity and Multiple Interactions.....	8
6.5. Signal Amplification – Step Up	9
6.6. Complex Formation In Vitro	9
6.7. Kinetics Series	11
Series A – Antigen-Antibody Interaction.....	11
Series B – Lectin-Lectin Interaction.....	12
6.8. Concentration Determination	13
7. Troubleshooting.....	13
Table 4 – Troubleshooting Issues and Solutions	13
8. Available Kits.....	14
9. References	15

1. Introduction

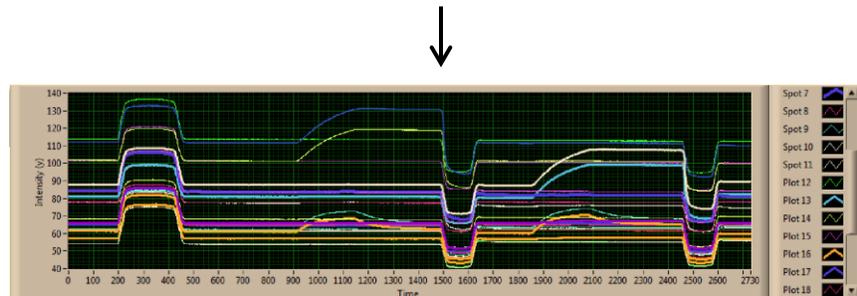
Surface Plasmon Resonance imaging (SPRi) technology can be used to measure molecular interactions and kinetics in real-time and with high-throughput. The Plexera Demo Chip has been developed and designed for use and demonstration with Plexera's *PlexArray*® Surface Plasmon Resonance imaging instrument, due to its high capacity and wide range of applications. This unique platform combines the microarray chip format with SPR, so that all users can easily and efficiently study multiple interactions between all kinds of molecules, in a multiplexed assay format.



Analyte and Ligand interaction (immobilized antibody and injected antigen shown)
This image represents one of many ligand spots on a Nanocapture® chip



Ligands are immobilized on a Nanocapture chip, allowing for high-throughput assays



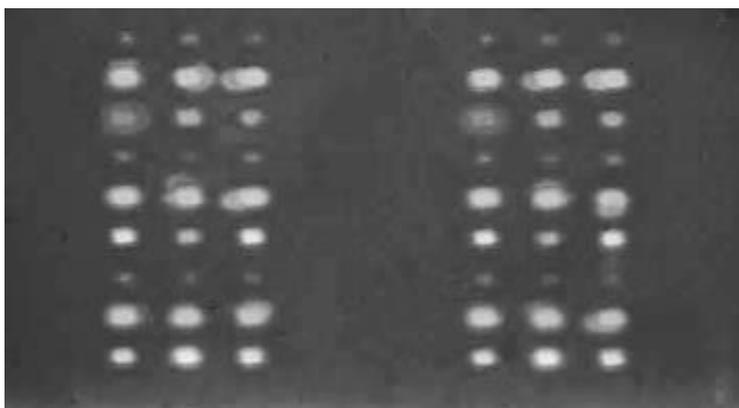
Binding of one analyte to many ligands can be observed in real time via the Plexera Instrument Control software, and further kinetic analysis can be subsequently performed

The Demo Chip has been printed with a variety of well characterized ligands, and typical binding assays can be easily performed using only a few sets of ligands and analytes. While the Demo Chip provides the user with a small array, the kit allows the user to become familiar with the *PlexArray* instrument, and explore the capacity of the high-throughput array format and capabilities of the chip. The Demo Chip has been tested for long term stability and resistance to a multitude of regeneration solutions and cycles. It can be used many times for binding assays without the need for replacement.

2. Demo Chip Kit Description

Below is an image of the Demo Chip mounted on the SPR instrument, and visualized using the Plexera Instrument Control Module. The standard Regions of Interest (**ROIs**) for the Demo Chip are visible in the image. On each Demo Chip, there are triplicates of the immobilized ligands. Thus, with every injection, statistically significant data set replicates are generated with identical experimental conditions.

Row	Column I	II	III
1	C-1	C-1	C-1
2	C-2	C-2	C-2
3	L-3	L-6	L-9
4	C-1	C-1	C-1
5	C-2	C-2	C-2
6	L-2	L-5	L-8
7	C-1	C-1	C-1
8	C-2	C-2	C-2
9	L-1	L-4	L-7



Maps of ligands and controls on the chip

Real image of ligands and controls on the array chip, seen through the instrument control software (only 2/3 triplicates shown)

Ligand	ROI
L-1	0
L-2	1
L-3	2
L-4	3
L-5	4
L-6	5
L-7	6
L-8	7
L-9	8

The Ligands L-1 through L-9 represent the immobilized ligands on the surface of the chip. Shown above are both a graphical interpretation of what you observe on the Plexera Instrument Control software, and a screenshot capture of the array duplicates as seen through the real-time imaging of the Instrument Control software.

The table on the left indicates how the Regions of Interest should be defined on the Instrument Control software. This will allow you to match the ROIs and your experimental results to the sample data provided later on in the manual under the “Typical Cases” section. Duplicate or triplicate spots on the array can also be selected as additional ROIs, providing replicate data sets.

3. Demo Chip Kit Components

The Demo Chip package contains one Nanocapture chip, printed with a variety of molecules. Additionally, analytes and the appropriate buffers are provided to demonstrate a multitude of binding profiles, calculate the kinetics of different molecular interactions, and clean the fluidics of the instrument (see **Table 1**). Enough analyte is provided to perform five replicates of each experiment (excluding Analyte A-6). See the following table for a detailed description of the contents of the Demo Chip Kit contents.

Table 1 – List of Kit Components

No	Item	Description	Directions for Use	Storage & Stability	Demo Data Fig
1	SPRi Chip	One Nanocapture chip printed with triplicates of 9 ligands	Load the flow cell with PBS, then mount on the SPR	4°C 6 months	N/A
2	Analyte 1 (A-1) ^a	30µl per vial Ready to use	Use with Assay Buffer #1	4°C 6 months	5 , 6 , 7
3	Analyte 2 (A-2) ^a	10µl per vial Ready to use	Use with Assay Buffer #1	4°C 6 months	5 , 6
4	Analyte 3 (A-3) ^a	10µl per vial Ready to use	Use with Assay Buffer #1	4°C 6 months	N/A
5	Analyte 4 (A-4) ^a	10µl per vial Ready to use	Use with Assay Buffer #1	4°C 6 months	3
6	Analyte 5 (A-5) ^a	10µl per vial Ready to use	Use with Assay Buffer #1	4°C 6 months	N/A
7	Analyte 6 (A-6) ^a	3µl per vial Ready to use	Use with Assay Buffer #1 – will inactivate Ligand L-3 spots	4°C 6 months	1
8	Analyte 7 (A-7) ^a	30µl per vial Ready to use	Use with Assay Buffer #2	4°C 6 months	2 , 4 , 7
9	Assay Buffer 1 ^{b, c}	40ml per tube 10x stock	For use with Analytes A-1 through A-6	4°C 1 month (after dilution)	1 , 5 , 6 , 7
10	Assay Buffer 2 ^b	40ml per tube 10x stock	For use with Analyte A-7	4°C 6 months	2 , 4 , 7 , 8
11	Wash Buffer ^b	40ml per tube 10x stock	Used for cleaning the fluidics of the instrument	4°C 6 months	N/A
12	Regeneration Buffer ^b	40ml per tube 10x stock	Use for regeneration cycles to remove bound analyte from the immobilized ligands after injections	Room temperature 6 months	N/A

- For analytes A-1 through A-7, spin down the contents of the vial with a centrifuge prior to use, and reference Section 6, Typical Cases, for amounts needed for each injection
- For Assay Buffers 1 and 2, as well as the Wash and Regeneration buffers, dilute the stock 10x concentration to 1x using deionized water prior to use
- Due to the shorter stability of Assay Buffer 1, you should not dilute the entire 10x stock at the buffer at once – instead, incrementally dilute portions of the stock as needed

4. Instructions

Table 2 – Demo Chip Setup

Step	Description	Details
1	Load the Flow Cell	<ul style="list-style-type: none"> Load the flow cell with assay buffer or PBS – will require 75µl Can blow out buffer with nitrogen and reload if initial loading doesn't fill at least ¾ of the flow cell
2	Mount the Chip	<ul style="list-style-type: none"> Place the slide in the housing of the <i>PlexArray</i> instrument Place 2-3 drops index fluid between the prism and the chip
3	Dilute the Sample	<ul style="list-style-type: none"> Dilute sample to 1ml using the appropriate buffer
4	Prime Flow Cell	<ul style="list-style-type: none"> Use the "Prime Flow Cell" function to flush assay buffer through the flow cell

	<ul style="list-style-type: none"> • Can use a high flow rate to remove any air bubbles
5	Blocking the Surface <ul style="list-style-type: none"> • Make sure that Assay Buffer 1 is primed in the system • Insert a long (20-30 minute) baseline step in the methods page at a slow flow rate (1-2μl/s) to complete the blocking of the chip surface

5. Procedures

This section quickly outlines how to establish a method for completing any of the experiments detailed in the Demo Chip Kit. Below is an example image of the “Method Setup” tab, and in the table below there are instructions on how to fill in the lines to create a protocol for the automated experimental process (see **Table 3**).

Table 3 – Method and Procedure Guidelines

Step	Description	Details
1	Add Analyte	<ul style="list-style-type: none"> • Add an Analyte line to the Method table • Define the location of the sample (BIG1-6 or SMALL1-6 for individual Eppendorf tubes, or A1-H12 for a 96 well plate)
2	Set Flow Rate/Time	<ul style="list-style-type: none"> • Choose the rate at which each analyte will be injected Standard: 4μl/second • Define the duration of the injection Standard: 230 seconds
3	Add Regeneration	<ul style="list-style-type: none"> • Add a regeneration cycle after each analyte injection Duration: 120 seconds
4	Add Baseline	<ul style="list-style-type: none"> • Can add a short baseline step between regeneration and the next injection Duration: 60-120 seconds • Can help establish a stable baseline to help data analysis
5	Number of Injections	<ul style="list-style-type: none"> • Define the number of injections you need to complete an experiment • Make sure that each injection is followed by regeneration
6	Save Data	<ul style="list-style-type: none"> • After the experiment is complete, save the .avi video file for analysis • Also save the .txt file for further analysis

Location	Name	Concentration (Mol/L)	Association Flow Rate (μ L/s)	Association Duration (s)	Dissociation Flow Rate (μ L/s)	Dissociation Duration (s)
BIG1	Analyte A-1	1.00E-08	4	230	4	30
4	Regen 1	---	4	120	0	0
BIG2	Analyte A-2	1.00E-08	4	230	4	30
4	Regen 1	---	4	120	0	0
0	Baseline Stabilization	---			4	120
BIG3	Analyte A-3	1.00E-08	4	230	4	30
4	Regen 1	---	4	120	0	0
0	Baseline Stabilization	---			4	120

6. Typical Cases

There are a variety of assays and experimental cases that can be demonstrated with the use of the Demo Chip Kit.

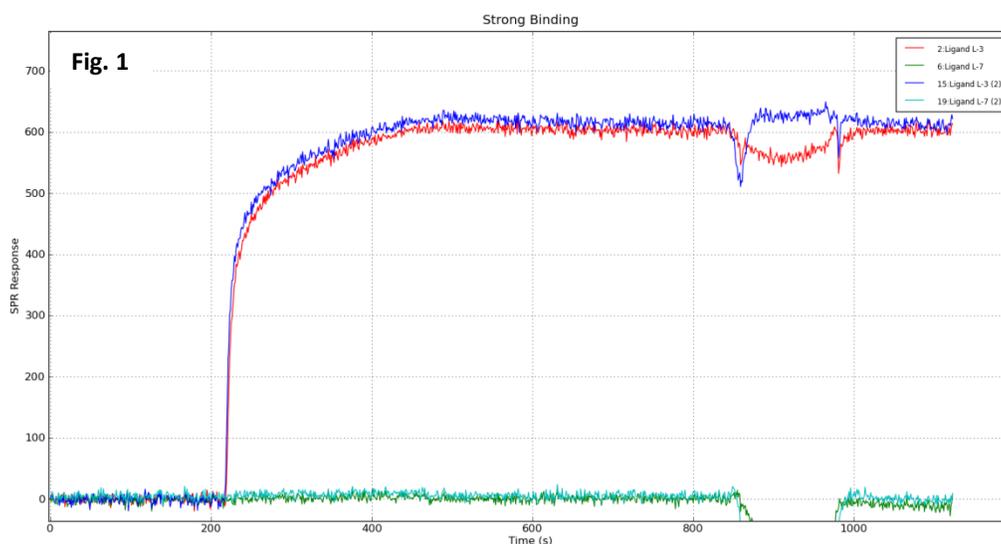
The most common and simple type is the one-to-one relationship experiment, which can be performed using any analyte provided in the Demo Chip package. For this assay, the typical analyte injection and regeneration cycle

should be employed. With one injection, binding to any of the multitude to ligands on the chip can be observed and recorded. You can observe real-time binding on up to 25 ROIs within the Instrument Control Panel, and using the data analysis software, you can subsequently determine the binding to any point on the array by measuring intensity changes from the recorded .avi video file of the experiment. This type of experiment can result in a variety of binding profiles and kinetics, depending upon which analytes are injected. Both weak and strong binding examples are shown below. It is important to note that some analytes, such as Analyte A-6, will very tightly bind to ligands on the chip, and cannot be regenerated without risking damage to the other ligands. As a result, Analyte A-6 should be the last injection performed on the chip to prevent tampering the results of other experiments.

There are also a handful of other useful assays that can be carried out on SPR, and that can be shown and executed using the contents of the Demo Chip Kit. Below are some results for different types of binding and experiments that can be performed and observed using the Demo Chip kit.

6.1. Strong Binding

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 1	Biotin-Streptavidin	Single	A-6 60kDa	1µl 16.67nM	4µl/s	L-3	2



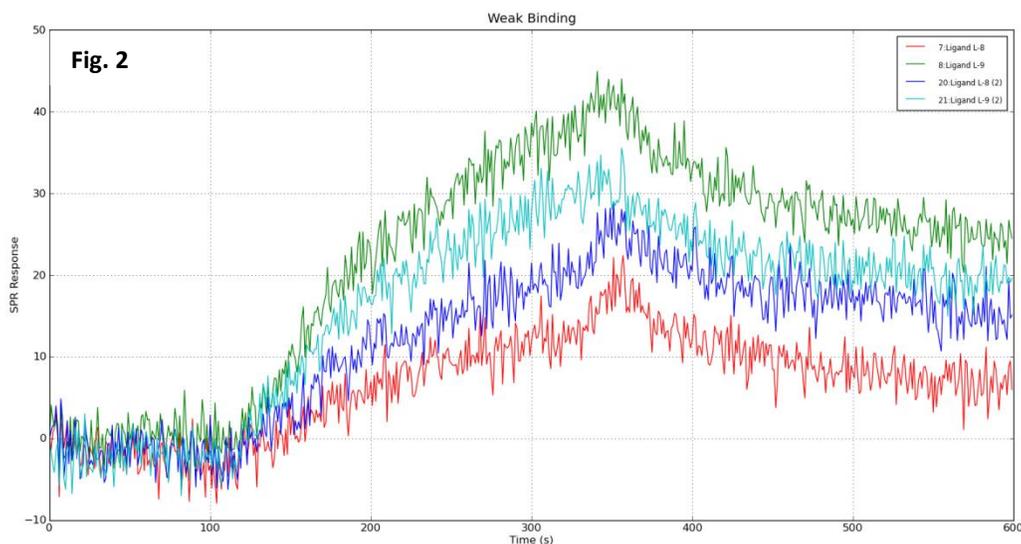
Strong binding is often characterized by a large increase in signal and a fast on-rate (the large uptick on the graph at just after 200 seconds), followed by a slow off-rate, and only a small decrease in signal during the dissociation phase of the injection. In some cases, one or more regeneration cycles will not be sufficient in stripping the analyte from the ligand on the chip. One such example, using Analyte A-6, is shown above.

It's important to note that after the regeneration step, the signal from the ligand does not return to the previous baseline signal established prior to the injection. This is due to strong covalent residue binding between the analyte and the immobilized ligand.

The affinity of this interaction is so strong, that it falls outside the measurable range of the SPRI instrument. However, Plexera can ensure that if this case is performed correctly, the user will observe an on-rate of greater than 10,000 1/(M•s), and an off-rate of less than 10⁻⁵ 1/s.

6.2. Weak Binding

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 2	Lectin-Lectin	Single	A-7 128kDa	2µl 15.6nM	4µl/s	L-8, L-9	7, 8



As opposed to the strong binding example given before, with this injection of Analyte A-7, the observed on-rate is slower, and the overall SPR signal is lower. Additionally, the off-rate is faster, resulting in a rapid signal decrease during the dissociation step. The binding difference between analyte A-1 and ligands L-8 and L-9 is quite apparent.

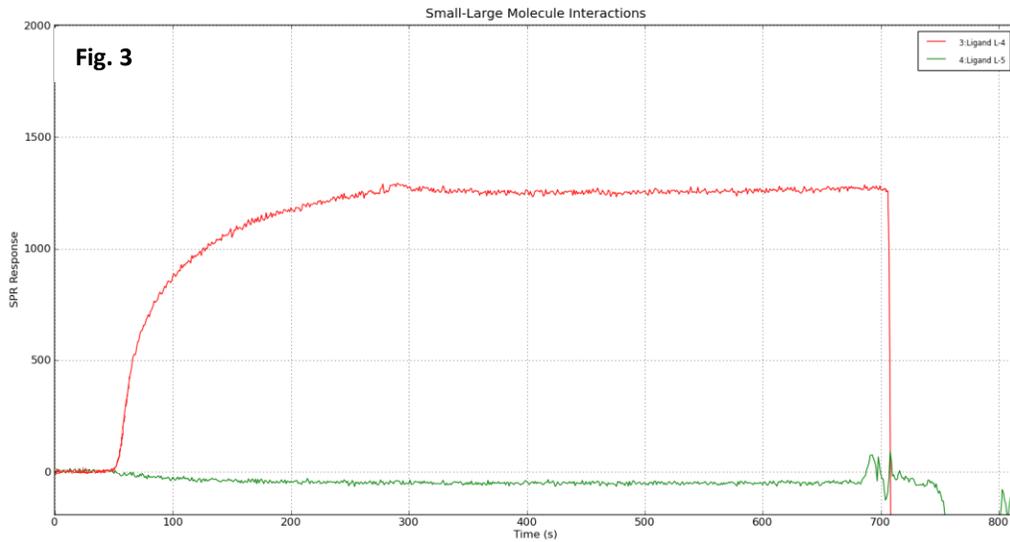
On this graph, please note the values on the Y axis. The apparent increase in signal noise is a result of the lower overall signal response during this experiment. Thus, the noise appears to be far more prominent than in the other Typical Cases data.

6.3. Small-Large Molecule Interactions

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 3	Small-Large Molecule	Single	A-4 66.6kDa	2µl 30nM	4µl/s	L-4	3

SPRi technology can allow users to measure the interactions between small molecules and large molecules. This can be accomplished by immobilizing the small molecule on the chip and injecting a protein, or by immobilizing the protein, and injecting the small molecule of interest.

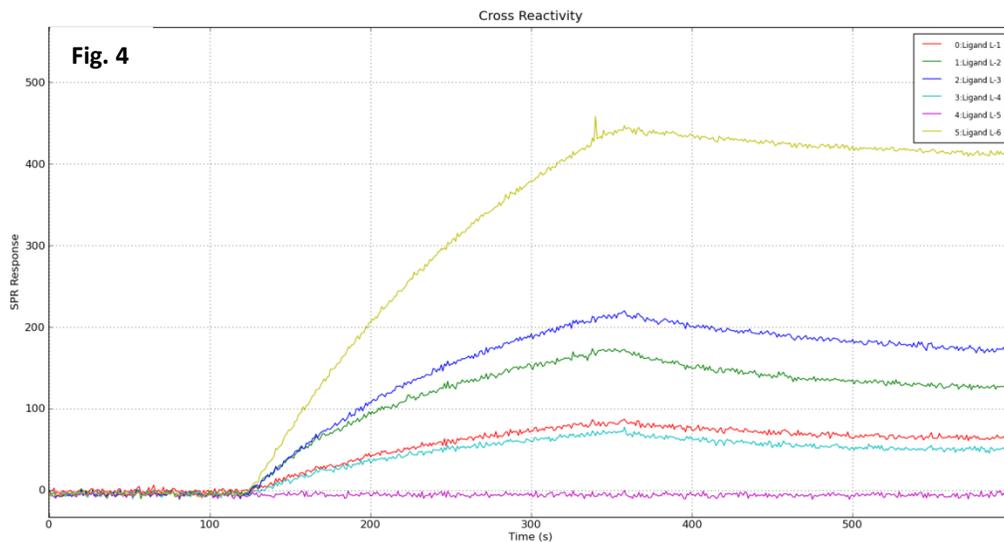
The issue presented in this model is that, when injected, small molecules do not have enough mass to cause any noticeable change in SPR signal. Additionally, not all small molecules will immobilize on a chip, and if they are able to immobilize, you risk the inactivation of the portion of the molecule that interacts with your analyte of interest.



To solve this issue, small molecules can be conjugated to larger ones, such as a carrier protein, to increase the overall mass of the complex, and to make it possible to observe an interaction between a small molecule, and a larger one via SPR imaging. One such interaction is demonstrated above. In this injection, a small molecule a mass of 184Da is conjugated to a larger molecule with a mass of 66kDa.

6.4. Cross Reactivity and Multiple Interactions

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 4	Lectin-Multiple	Single	A-7 128kDa	2µl 15.6nM	4µl/s	L-1, L-2, L-3, L-4, L-6	0, 1, 2, 3, 5



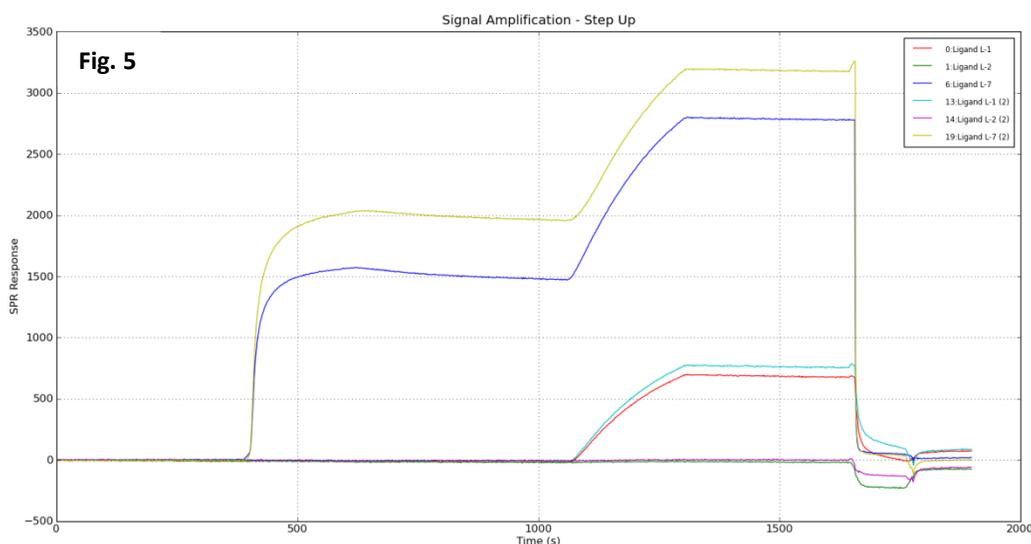
When performing a simple one-to-one assay, it can be useful to identify a variety of ligands to which one analyte will bind. Due to the high-throughput capabilities of the *PlexArray* Surface Plasmon Resonance instrument, one injection of an analyte will provide binding data for all the ligands immobilized on the Nanocapture chip. This can

be useful for determining both cross reactivity and multiple interactions of an analyte to a variety of proteins or molecules.

While the injection of Analyte A-7 (shown above) shows considerable binding to a multitude of ligands on the chip, the binding profiles to each ligand are unique and comparable.

6.5. Signal Amplification – Step Up

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 5	Antigen-Antibody	Double	A-1, A-2 150, 150kDa	2µl (ea) 13.3nM	4µl/s	L-7	6

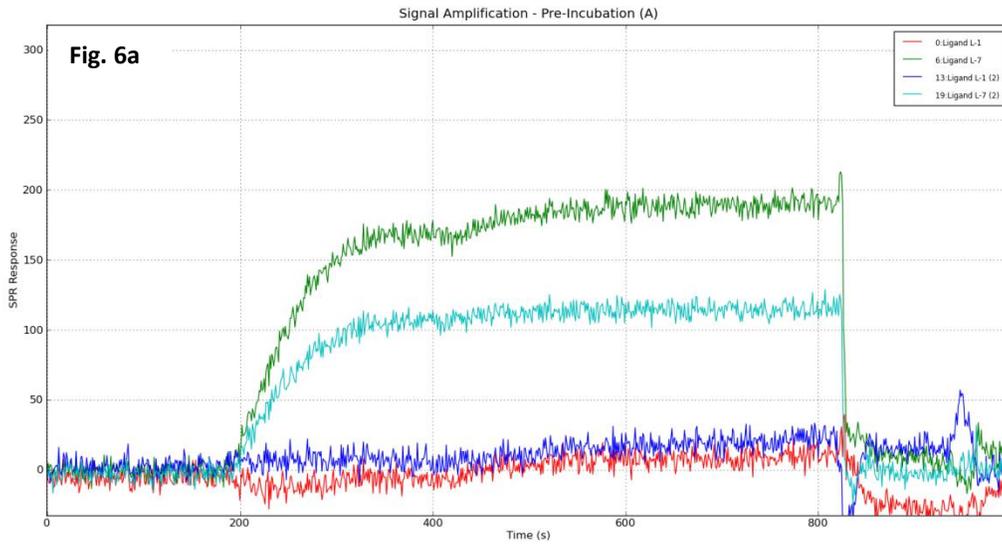


Signal strength from an analyte can sometimes be relatively weak, depending on a multitude of factors, such as analyte concentration and kinetics of the ligand-analyte interaction. The signal strength from an experiment can be amplified with a secondary binding injection method. Start by injecting one analyte of interest, and then without regeneration, inject another analyte with which the first interacts. This creates a larger complex mass that is bound to the ligand. This secondary ligand binding can provide a larger SPR signal as a result. To perform this assay, you must minimize the dissociation time between the injections, so that you maximize the amount of the initial analyte that remains bound to the ligand. The figure above clearly demonstrates the binding pattern through subsequent injections.

6.6. Complex Formation In Vitro

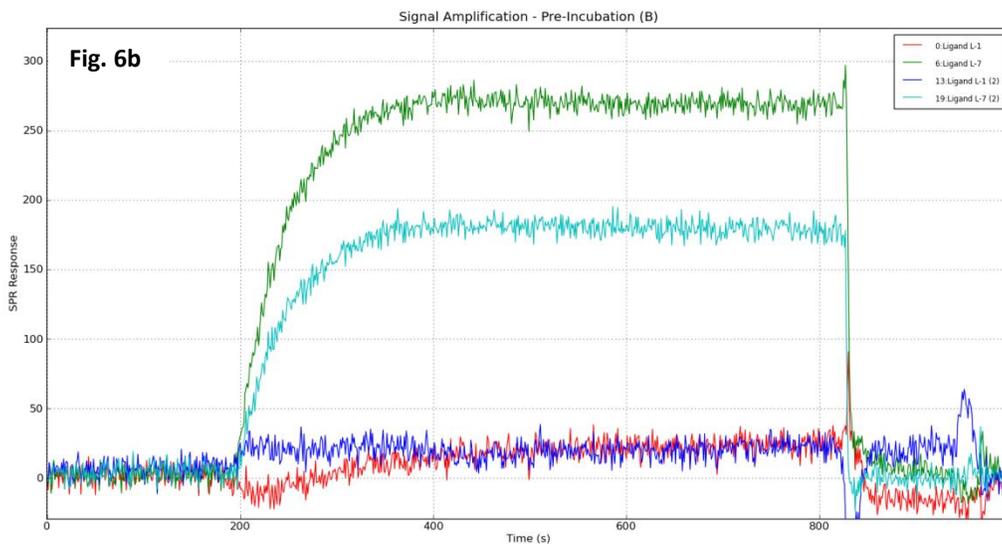
Alternatively, instead of using subsequent injections to increase the signal of an analyte-ligand interaction, you can also create a larger analyte mass by using a pre-incubation period, involving two or more analytes, prior to the injection.

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 6a	Antigen-Antibody	Single (1/2)	A-1 150kDa	2µl 13.3nM	4µl/s	L-7	6



In the first graph, only Analyte A-1 is injected, and the SPR response is roughly 175 RU. The second graph depicts a separate injection, in which equal amounts of Analytes A-1 and A-2 are pre-incubated for a period of 30 minutes prior to injection. Due to the interaction between the two analytes during the incubation period, the total mass of the final analyte complex increases, and thus increases the resulting SPR signal. As a result, in the second experiment and graph, the SPR response from the injection increases to around 275 response units. This shows that by using a second analyte to increase the overall mass, smaller signals can be more easily detected, as long as the second analyte will not interfere with the binding of the complex to the ligand via competition.

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs
Fig. 6b	Antigen-Antibody	Single (2/2)	A-1 + A-2 300kDa total	2 μ l (ea) 13.3nM	4 μ l/s	L-7	6



It is often useful to inject both analytes individually prior to the assay. This is to ensure that there is not identical binding to the ligand of interest, and to establish a norm for SPR response from the injection. If the pre-incubated

analyte sample gives a lower final signal, or shows no binding, there is competitive binding between the two analytes and your region of interest on the chip. Therefore, the *PlexArray* system and chip gives the user flexible options for performing competition, inhibition, and blocking assays.

6.7. Kinetics Series

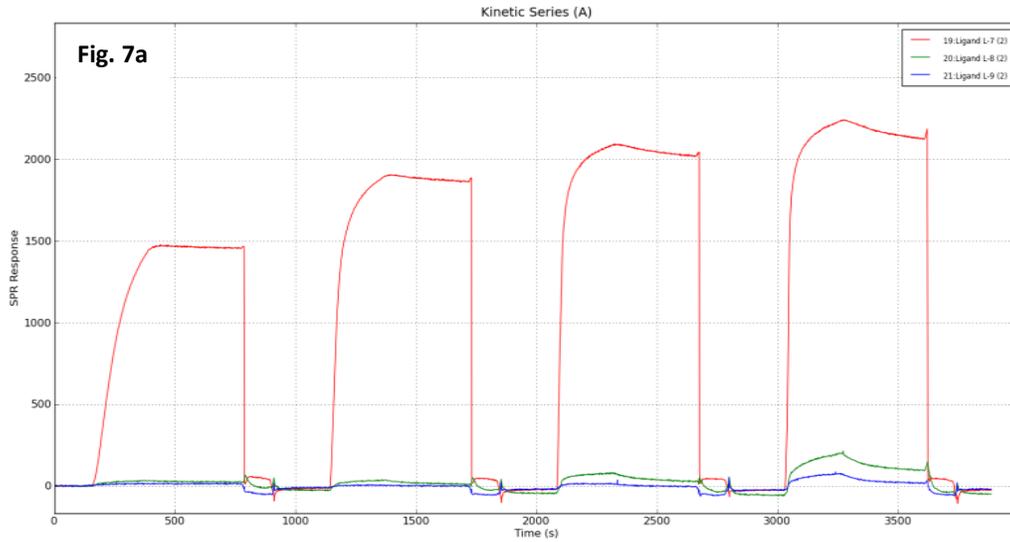
Perhaps the most beneficial use of the SPR instrument is to determine the kinetics of a binding interaction between an injected analyte and immobilized ligands on the chip. Determining kinetic data can be demonstrated using the Demo Chip and the Plexera Data Analysis Module. Multiple injections of varying concentrations are needed of the same analyte to determine the kinetics of the binding to the ligands on the chip. The analyte of interest must be prepared in a dilution series. The standard procedure is to inject four different concentrations, differing by a factor of three (example: 2.23, 6.68, and 20.04, and 60.12nM). Kinetics can be determined with as few as three injections, but additional concentrations reduce error in determining kinetic data.

Shown below are two different dilution series used for kinetics and affinity determination. The first graph (example A – Antibody-Antigen), depicts a series using Analyte A-1 in Assay Buffer #1, while the second graph (example B – Lectin-Lectin) uses a dilution series of Analyte A-7 in Assay Buffer #2.

*While there is listed example data for the solved kinetics for the following assays, there can be a wide variance in solved kinetic values among labs and instruments. So while any solved kinetics should be somewhat similar to the values provided in this manual, variance up to one order of magnitude is quite possible.

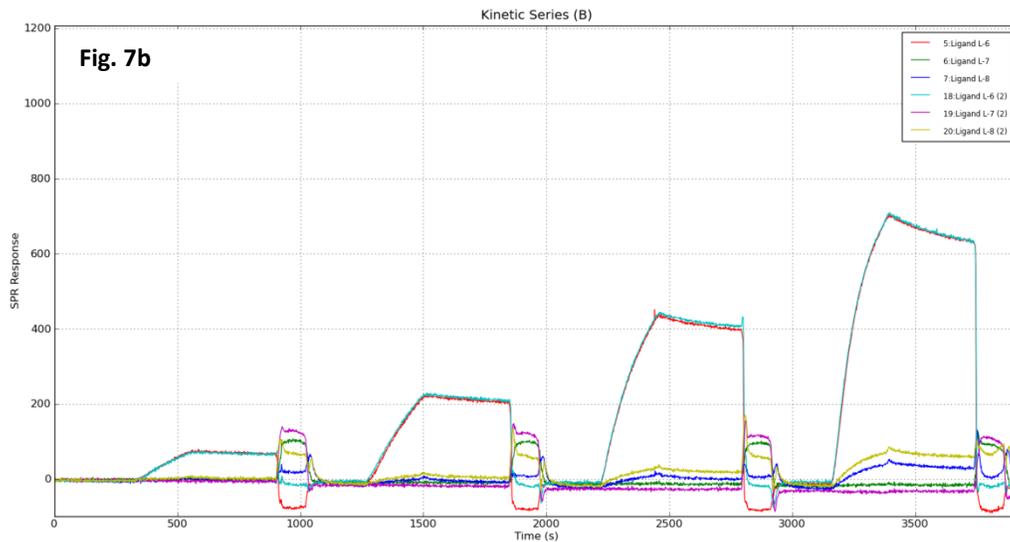
Series A – Antigen-Antibody Interaction

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs	k_a^*	k_d^*	K_D^*
Fig. 7a	Antigen-Antibody	Series	A-1 150kDa	0.3µl 2.23nM	4µl/s	L-7	6	2.6×10^6	1.9×10^{-4}	7.2×10^{-11}
				1µl 6.68nM						
				3µl 20.04nM						
				9µl 60.12nM						



Series B – Lectin-Lectin Interaction

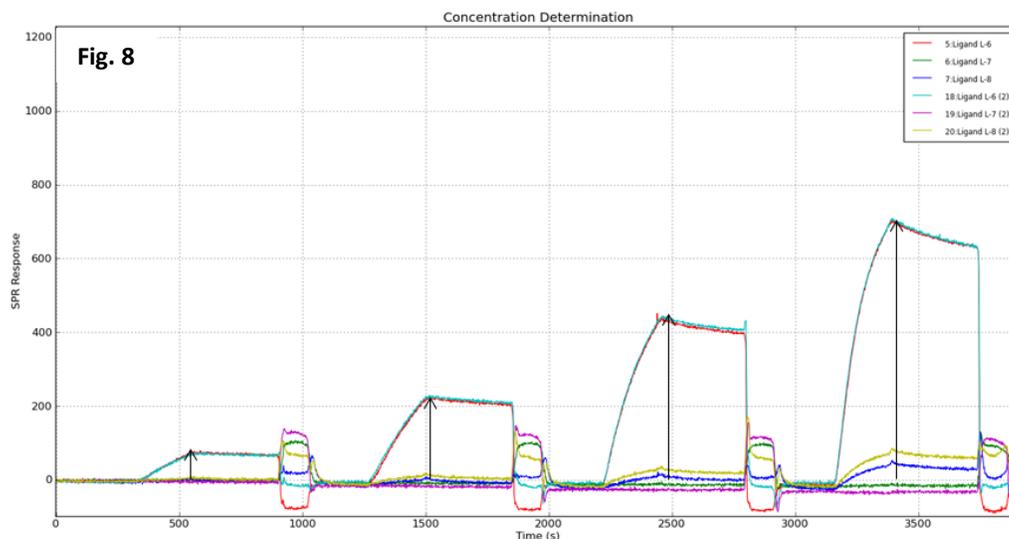
Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs	k_a^*	k_d^*	K_D^*
Fig. 7b	Lectin- Lectin	Series	A-7 128kDa	0.27 μ l 2.23nM	4 μ l/s	L-6	5	1.8×10^5	4.2×10^{-4}	2.3×10^{-9}
				0.8 μ l 6.68nM						
				2.4 μ l 20.04nM						
				7.2 μ l 60.12nM						



These experiments resulted with successive injections of different concentrations of an analyte. This data can then be used, combined with the Plexera Data Analysis Module, to determine the affinity of an interaction between a ligand and analyte. It can also be useful to use increasing concentrations until saturation in signal is reached. This is identified by two or three successive injections of increasing concentration reaching a similar or identical index change value (on the Y axis). This saturation is becoming visible in the 3rd and 4th injections of Series A.

6.8. Concentration Determination

Demo Data	Interaction Type	Injection	Analyte/ MW	Amount Injected	Flow Rate	Interacting Ligands	Interacting ROIs	Signal Response
Fig. 8	Lectin-Lectin	Series	A-7 128kDa	0.27µl 2.23nM	4µl/s	L-6	5	80
				0.8µl 6.68nM				235
				2.4µl 20.04nM				450
				7.2µl 60.12nM				715



If the concentration of a particular molecule in a solution is unknown, SPR can be used to determine the concentration. You can perform a concentration series injection of the molecule of interest (Analyte A-7 shown above) and plot the signal response against the concentration of the analyte injection. After finding this correlation, you can perform an injection of the unknown concentration, and use the solved correlation value to determine the unknown concentration.

7. Troubleshooting

Table 4 – Troubleshooting Issues and Solutions

Issue	Causes	Solution
Air in flow cell	<ul style="list-style-type: none"> Air present in analyte injections Air present in the assay buffer 	<ul style="list-style-type: none"> Stop (interrupt) the experimental run Using “Load and Prime Flow Cell”, try a short, fast

	<ul style="list-style-type: none"> The instrument de-gasser is not activated 	<ul style="list-style-type: none"> flow rate, such as 20-40μl/sec for a period of 3-5 seconds Repeat this short pulse a few times to try to remove air You can also try a long (20-30 min) baseline stabilization at a slow rate (1μl/s) Repeat as many times as necessary
Jam detection	<ul style="list-style-type: none"> Auto-sampler needle impacting a surface during a run Some sort of object in the way of the auto-sampler's path 	<ul style="list-style-type: none"> Watch a portion of the experiment run to look for the cause of the jam Look for any loose objects around the auto sampler, such as lids of microtubes Make sure all tubes and well plates are secure and in place
Clog in system	<ul style="list-style-type: none"> Buildup of protein or other material within the tubing of the instrument 	<ul style="list-style-type: none"> Look to make sure that fluid is exiting the waste line denoted "0" Remove the slide from the housing Using a thin pin or paper clip, insert it into the channel that pumps buffer into the flow cell to try to alleviate the clog If the problem is not fixed, contact technical support
High background signal	<ul style="list-style-type: none"> Insufficient blocking of the surface after ligand spotting 	<ul style="list-style-type: none"> Do a long (30 minute) baseline injection of Assay Buffer #1 at a rate of 4μl Repeat until a steady baseline signal is observed
No signal	<ul style="list-style-type: none"> Inactivity of ligand(s) Inactivity of analyte(s) Wrong assay buffer 	<ul style="list-style-type: none"> Ensure that you are using the appropriate assay buffer Make sure that the chip and analytes are still within the activity date listed in the manual
Too much signal	<ul style="list-style-type: none"> Wrong amount of analyte injected 	<ul style="list-style-type: none"> Double check that you are using the correct amount of analyte as specified in the manual
Poor reproducibility	<ul style="list-style-type: none"> Wrong amount of analyte injected Wrong assay buffer Inactivity of ligand(s) Inactivity of analyte(s) 	<ul style="list-style-type: none"> Ensure that you are using the appropriate assay buffer Make sure that the chip and analytes are still within the activity date listed in the manual Double check that you are using the correct amount of analyte as specified in the manual

If the user continues to experience issues after consulting with this table, please contact Plexera technical support for additional assistance (cro@plexera.com).

8. Available Kits

Product	Content	ID No	Price	Detail
PlexArray® Demo Kit I	<ul style="list-style-type: none"> Included with instrument purchase Complete contents of Demo Kit IV 	BG-DE-K001	Included with Instrument	PDF manual
PlexArray® Demo Kit II	<ul style="list-style-type: none"> 1x Demo Chip Manual 	BG-DE-K002	Inquire for price	PDF manual

PlexArray® Demo Kit III	<ul style="list-style-type: none"> • 1x Demo Chip • Assay buffers • Analyte reagents • 5 reactions per analyte • Manual 	BG-DE-K003	Inquire for price	PDF manual
PlexArray® Demo Kit IV	<ul style="list-style-type: none"> • 2x Demo Chip • Assay buffers • Analyte reagents • 10 reactions per analyte • Manual 	BG-DE-K004	Inquire for price	PDF manual

9. References

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Array + Chip + SPRi = Screening any Analyte and Ligand Through Biointeractions

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