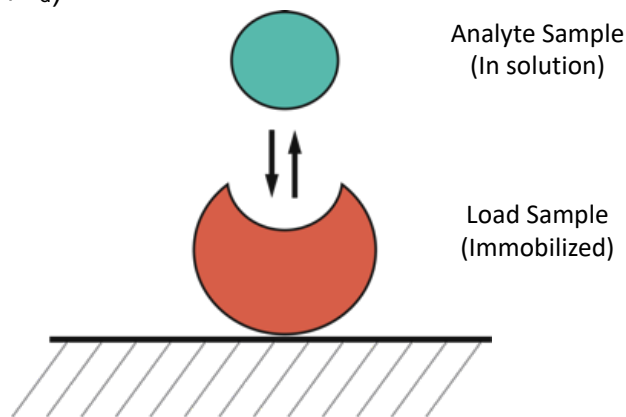


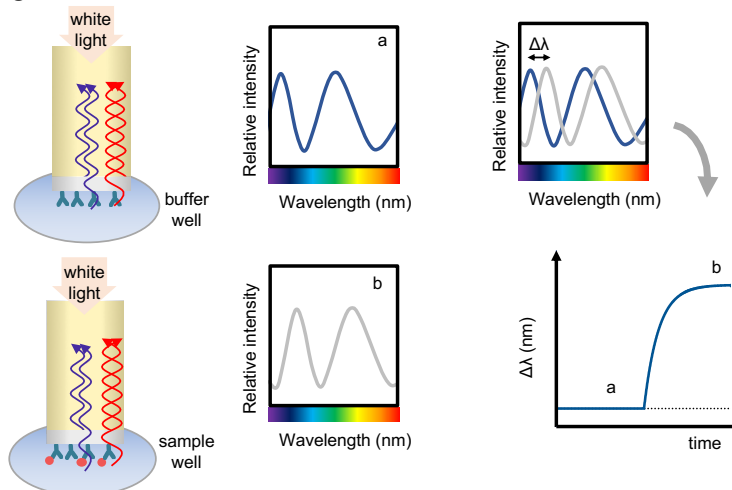
Octet RED384 Getting Started

Introduction

The Octet RED384 is an instrument for Biolayer Interferometry (BLI), an optical technique for measuring macromolecular interactions by analyzing interference patterns of white light reflected from the surface of a biosensor tip. BLI experiments are used to determine the kinetics and affinity of molecular interactions. In a BLI experiment, one molecule (the **Load Sample**) is immobilized to a Dip and Read Biosensor and binding to a second molecule (the **Analyte Sample**) is measured. A change in the number of molecules bound to the end of the biosensor tip causes a shift in the interference pattern that is measured in real-time. BLI can be used to measure kinetic binding constants (k_a , k_d) and equilibrium binding constants (affinity, $K_a = 1/K_d$).



Response is measured as a nm shift in the interference pattern and is proportional to the number of molecules bound to the surface of the biosensor. Response is recorded and displayed on a sensogram in real time.



Sample Preparation Guidelines

Recommended concentration ranges

Load Sample (to be immobilized) concentration	10-50 µg/ml (~µM range)
Analyte concentration	0.01-100xK _D (0.1-10xK _D)

Sample Plate Volume Recommendations

96-well	200 µl
384-well	80-120
384-well tilt bottom	60

Buffers

Many buffers are compatible with BLI, so it's usually a **good idea to start with a buffer system in which your proteins are well behaved**. The sample used for the association phase should be in a buffer identically matched to that used for the baseline and dissociation phase (especially when a buffer component has a high refractive index, such as DMSO). Immobilized load sample should also be in the same buffer, if possible. Addition of 0.02% (0.005%-0.1%) Tween 20 or other detergent, is usually necessary as it can help to prevent non-specific binding, a frequent problem in BLI experiments. ForteBio sells a buffer they call their Kinetic Buffer (PBS+ 0.02% Tween20, 0.1% BSA, 0.05% sodium azide).

Supplies

For each experiment, you'll need to bring ForteBio Biosensors, at least 2 black microplates (one for soaking sensors and at least one for samples and reagents), an empty biosensor tray to use as a working tray, and general lab supplies for liquid handling (pipettes, tips, tubes, gloves, etc.).

OCTET general supplies (plates)**

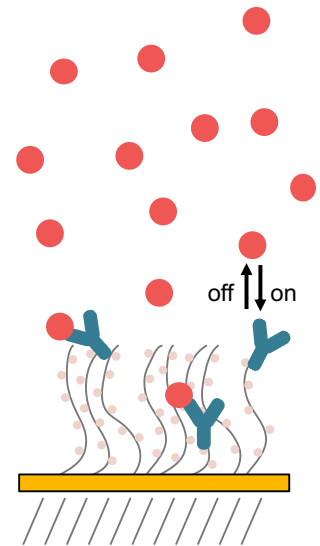
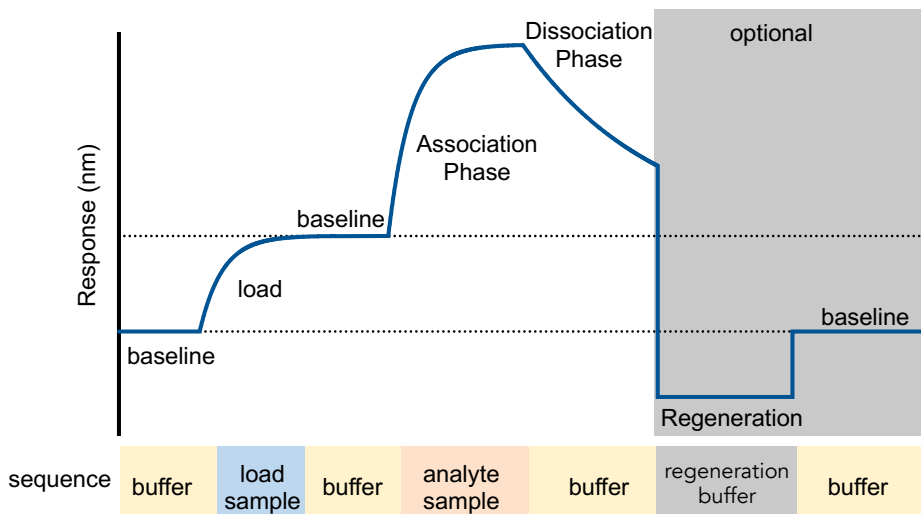
	Part Number
Greiner Bio-One 96-well black flat-bottom PP, 200 µL	655209 (VWR 82050-784)
Greiner Bio-One 384-well black flat-bottom PP, 80-120 µL	781209 (VWR 82051-318)
ForteBio 384-well black tilted-bottom PP, 60 µL	18-5080

ForteBio Dip and Read Biosensors[‡] (96/tray)

	Part Number
Streptavidin (SA) biosensors	18-5019
anti-His (HIS2) biosensors	18-5114
anti-His (HIS1K) biosensors, NEW -for kinetics	18-5120
Ni-NTA (NTA) biosensors	18-5101
anti-GST biosensors	18-5096
Super-Streptavidin (SSA) biosensors	18-5057
Protein A biosensors	18-5010

** Only **Greiner Bio-One brand, black microplates** or ForteBio plates are recommended for the sample and reagent plate.

‡ See ForteBio website for more sensor types: <http://www.fortebio.com/biosensor-types.html>.



Experimental Design Tips

- Don't overload the immobilized molecule
- The same well of buffer should be used for the baseline and dissociation phase, if interstep correction is performed
- For small molecule work, use Super-Streptavidin sensors and quench with biocytin (biotinyl-lysine at 10 $\mu\text{g/ml}$)
- Use reference subtraction. There are several types.
 - Reference well –has immobilized load sample and no analyte during association
 - Parallel Reference sensor –has *no load sample* immobilized and is matched for analyte concentration.
 - Double Reference –uses both reference well and parallel reference sensor
 - Custom Reference –any sensor can be designated as a reference and subtracted from any sample sensor (can't be used for double referencing)
- Experiments should be less than 3 hours (10% volume loss ~ 3.5 hours at 30C)

Starting the Instrument

- The instrument should be left on at all times.
- Start the Octet Data Acquisition Software (current version 10.0 on the Desktop)
 - Wait for initialization
 - Follow Experiment Wizard
 - select New Kinetics Experiment or open a method file
- Open the instrument door with Present Stage button in the software (green eject button). Do NOT pull on the thing that looks like a handle.

Setting up a Kinetics Experiment

1. Plate Definition

- a. Select data acquisition mode. Read Head: 16 channel or 8 channel
 - 16 channel mode uses up to 16 sensors and moves in 2 column increments
 - 8 channel mode uses up to 8 sensors and can move in 1 column increments
- b. To modify plate format, select Modify Plate
 - i. Plate 1= Sample Plate, Plate 2= Reagent Plate
 - ii. choose 96-well or 384-well format
- c. Select a well or wells to define (shift-click to select all wells in column on 96-well or alternating wells on 384-well plate)
- d. Right-click to pull up Sample Type Menu and choose type for the selected wells.
 - i. Select "Set Well Data" from the Sample Type Menu to add information or fill in the Plate Data tables.
 - ii. A molar concentration for the samples is required for fitting.
 - iii. include a zero concentration of analyte (for a reference well) to correct for drift in the baseline (almost always REQUIRED for NTA sensor type)

Tab 1. Plate Definition.

The screenshot shows the 'Basic Kinetics Experiment' software interface. The 'Plate Definition' step is active, showing a grid for defining wells and a table for well data. The grid is divided into Plate 1 (96 wells) and Plate 2 (96 wells). The table shows the following data:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
B A1			Buffer				
B B1			Buffer				
B C1			Buffer				
B D1			Buffer				
B E1			Buffer				
B F1			Buffer				
B G1			Buffer				
B H1			Buffer				
L A2	biotinylated protein X		Load	20			
L B2	biotinylated protein X		Load	20			
L C2	biotinylated protein X		Load	20			
L D2	biotinylated protein X		Load	20			
L E2	biotinylated protein X		Load	20			
L F2	biotinylated protein X		Load	20			
L G2	biotinylated protein X		Load	20			
L H2	biotinylated protein X		Load	20			
B A3			Buffer				
B B3			Buffer				
B C3			Buffer				
B D3			Buffer				
B E3			Buffer				
B F3			Buffer				
B G3			Buffer				
B H3			Buffer				
A4	protein Y		Sample	100	10000	10	
B4	protein Y		Sample	50	10000	5	
C4	protein Y		Sample	25	10000	2.5	
D4	protein Y		Sample	12.5	10000	1.25	
E4	protein Y		Sample	6.25	10000	0.625	
F4	protein Y		Sample	3.125	10000	0.3125	
G4	protein Y		Sample	1.563	10000	0.1562	
H4	protein Y		Sample	0	10000	0	reference well

2. Assay Definition

- a. Plate 1 and Plate 2 show plate layouts (step back to Plate Definition to modify)
- b. Create a list of steps in the Step Data List
 - i. sample steps (s):
 - baseline 60-300
 - loading 120-600
 - (quench) 60-120 optional quench (eg. Biocytin on SA or SSA)
 - association 60-600
 - dissociation 60-600
 - ii. shake speed 1000 rpm
- c. Create an assay (a group of ordered steps with plate information)
 - i. Select a column from a plate and select a step in Step Data List
 - ii. Double-click or click Add... to add a step to Assay Step List
 - iii. Select the sensor type for the assay
 - iv. To create new Assays, click New Assay or select all steps in Assay 1 and click replicate. Modify sample column as needed.
- d. Assign Parallel Reference Sensors (recommended to measure non-specific binding)
 - i. add two more columns of buffer to the sample plate
 - ii. select all steps in assay 1
 - iii. click replicate
 - iv. change the load step to one of the empty buffer wells
 - v. change the baseline before association and the dissociation to the other empty buffer well

Tab 2. Assay Definition.

The screenshot shows the 'Basic Kinetics Experiment' software interface. The 'Assay Definition' step is active. On the left, there are two 96-well plate layouts. Plate 1 shows a 4x3 grid of wells in columns 1-3, rows A-D, with a legend for 'Assayed samples' (shaded) and 'Unassigned samples' (white). Plate 2 shows a 4x3 grid of wells in columns 1-3, rows A-D, with a legend for 'Assayed reagents' (shaded) and 'Unassigned reagents' (white). On the right, the 'Step Data List' table is visible, containing the following data:

Name	Time	Shake speed	Type	Threshold
Baseline	60	1000	Baseline	<input type="checkbox"/>
Loading	120	1000	Loading	<input type="checkbox"/>
Association	300	1000	Association	<input type="checkbox"/>
Dissociation	600	1000	Dissociation	<input type="checkbox"/>

Below the Step Data List is the 'Assay Steps List' table, which contains the following data:

Assay No.	Sample	Plate	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	1	Baseline	Baseline	SA (Streptavidin)		
1	2	2	Loading	Loading	SA (Streptavidin)		
1	3	3	Baseline	Baseline	SA (Streptavidin)		
1	4	4	Association	Association	SA (Streptavidin)		
1	5	3	Dissociation	Dissociation	SA (Streptavidin)	0:20:00	

3. Sensor Assignment

- a. This step tells you where to put sensors in the Sensor Tray.
 - i. You should always check the box marked **Replace sensors in tray after use**. This will return sensors to the tray after your experiment (and prevent them from clogging the instrument, as there is a design flaw in the sensor discard shoot).
 - ii. Plate 1 (and 2) indicate wells that each sensor group will enter in an assay.
 - iii. Sensor type should match those from the Assay Definition.
- b. Fill a sensor tray with sensors in the marked positions and place 96-well plate underneath with soaking buffer (usually running buffer) in the wells with sensors.

Tab 3. Sensor Assignment.

The screenshot shows the 'Basic Kinetics Experiment' software interface. The 'Sensor Assignment' step is active, indicated by a red '3' in the top navigation bar. An information icon (i) provides instructions: 'In this step, sensors are assigned to samples. If you have a partial sensor tray it can be accommodated by selecting the missing sensors and clicking 'Remove'. Only the first sensor tray can be a partial tray. Right click to assign a sensor type to selected sensors.'

The 'Sensor Tray' section has a checkbox checked for 'Replace sensors in tray after use'. Below it is a 12x8 grid (wells 1-12, A-H). A legend indicates that white squares represent 'Unassigned sensors' and squares with a cross represent 'Missing sensors'. Buttons for 'Remove', 'Fill', 'Fill Plate', and 'Print...' are present.

The 'Plate 1 (96 wells)' section shows a 12x8 grid (wells 1-12, A-H). A legend indicates that white circles represent 'Unassigned samples'. Buttons for 'Remove', 'Fill', 'Fill Plate', and 'Print...' are present.

A table on the right lists the sensor assignments for wells A2 through H2:

Well	Sensor Type	Lot Number	Information
A2	SA (Streptavidin)		
B2	SA (Streptavidin)		
C2	SA (Streptavidin)		
D2	SA (Streptavidin)		
E2	SA (Streptavidin)		
F2	SA (Streptavidin)		
G2	SA (Streptavidin)		
H2	SA (Streptavidin)		

4. Review Experiment
 - a. Step through the assay steps to review

Tab 4. Review Experiment.

Assay No.	Sample	Plate	Step Name	Step Type	Sensor Type	As:
1	1	1	Baseline	Baseline	SA (Streptavidin)	
1	2	2	Loading	Loading	SA (Streptavidin)	
1	3	3	Baseline	Baseline	SA (Streptavidin)	
1	4	4	Association	Association	SA (Streptavidin)	
1	5	3	Dissociation	Dissociation	SA (Streptavidin)	0:

5. Run Experiment
 - b. Select location for data (choose your folder inside your lab folder)
 - c. Enter experiment run name (avoid using the default name Experiment_1)
 - d. Set the Run Settings
 - i. Check all boxes except for “Present Stage at end of experiment” which should be unchecked unless you will be present at the end of your experiment
 - ii. Set experiment delay for soaking and equilibration time (10 minutes)
 - iii. Set temperature (30C minimum)
 - e. Advanced Settings
 - i. Set Sensor Offset: 4 mm for standard well volumes (see table)
 - ii. Set Acquisition Rate: Standard Kinetics (5Hz, avg by 20)
 - f. Save method file if using later
 - g. Start Run by pressing Go button

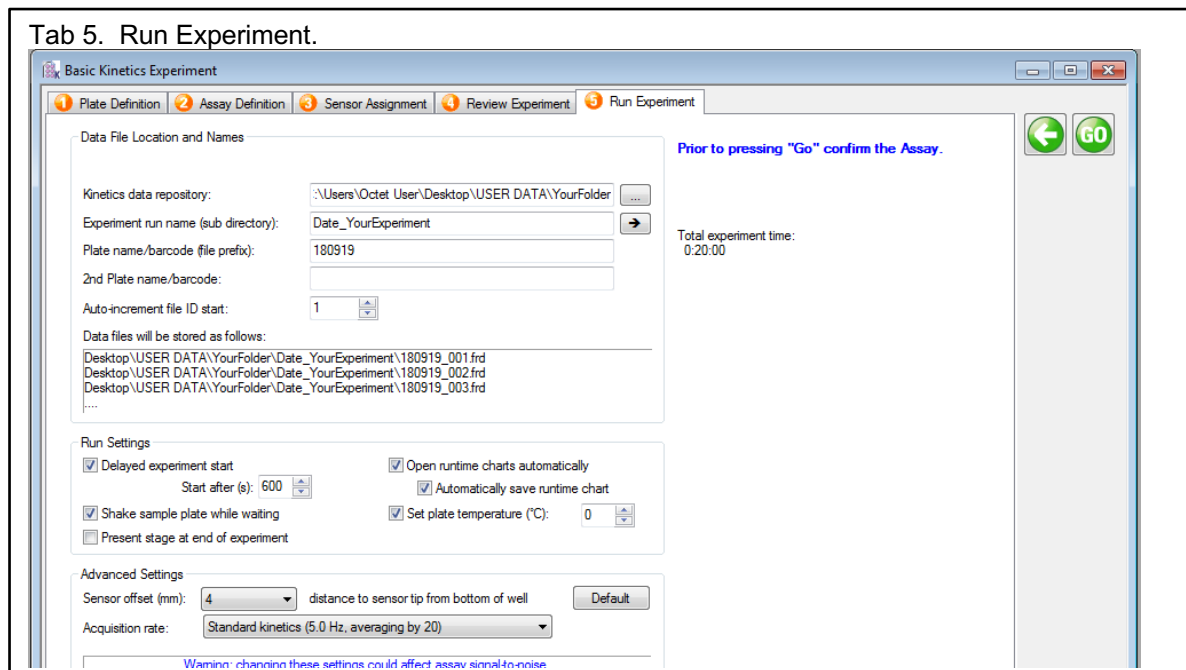


Table of Recommended sensor offsets by well volume (from Octet User Manual)

Table 2.3 Sensor offset and well volumes for Octet RED384 & QK384

Sensor Offset (mm)	Recommended Minimum Fill Volume (μ l)		
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (ForteBio, 384TW)
3	200	80	40
4	200	80	60
5	225	100	80
6	250	120	100
7	300	130	100

Kinetic Data Analysis

With the release of version 10.0 of the Octet software, ForteBio has released a fully-functional new analysis software package. **Data Analysis HT 10.0** has several new features that improve on the capabilities of original Data Analysis software. The Data Analysis HT software is the recommended choice for all new users. The legacy software, Data Analysis 10.0 is still available for use.

Data Analysis HT 10.0

new features:

- Data from multiple plates and/or experiments can be combined into one analysis
 - More flexible reference subtraction options for kinetic analysis
 - One-click raw data export into csv table
 - Customizable report format
-
1. Open the Data Analysis HT Software
 2. Select Data
 - a. Click Explore in the Icon Bar to view data folders
 - b. Drag an Octet data folder to the Experiment Builder
 - i. More than one data file can be combined into one analysis by dragging additional files into the Overlay section (must have the same number of steps and step lengths)
 - ii. Octet data can also be Appended to the beginning or end of a data set
 - to combine data with different step sizes
 - for experiments with steps performed in different files (such as immobilization and association performed as different experiments)
 3. Process Data
 - a. Click Preprocess from the Operations Section of the Icon Bar
 - b. Assign Reference Sensors (sensors to which no ligand is immobilized)
 - i. Go to Reference Sensor Tab
 - ii. Click on column number or drag to select reference sensors
 - iii. Right click on selection
 - iv. Set Sensor Type to Reference Sensor (sensor icon will change to diamond)
 - v. Right click on plate
 - vi. Go to Subtract Reference and choose subtraction method
 - In Rows (selects a reference from the same row as the sample)
 - vii. Scroll down to see multiple plates and repeat for each plate

- viii. Check the table at the bottom to see the sensor subtraction formula used for each sensor
- c. Assign Reference Samples (samples with zero concentration of analyte)
 - i. Go to Reference Sample Tab
 - ii. Click (or CTRL-click) to select well(s) for reference samples (zero concentration)
 - iii. Right click on selection
 - iv. Set Reference to Reference Sample Wells
 - v. Right click on plate
 - vi. Go to Subtract Reference and choose subtraction method
 - In columns (selects a reference well from the same column as the samples)
 - vii. Scroll down to see multiple plates and repeat for each plate
 - viii. Check the table to see the well subtraction formula for each sensor
- d. Data Corrections
 - i. Go to Data Correction Tab
 - ii. Align Y axis to average of baseline step (default is the last 5 seconds of the baseline)
 - iii. Inter-step correction
 - iv. This step corrects for system artifacts (optical artifacts from buffer mismatch, etc.)
 - v. Choose a step to align (try dissociation first)
 - Baseline before association and dissociation steps must be performed from the same well of a sample plate
 - Should not be performed with very fast on-rates as kinetic data may be lost
- e. Export Processed data: click on Processed Data in Export Section of the Icon Bar to export data in a csv format for graphing in other programs.**

4. Kinetic Analysis

- a. Fitting Parameters
- b. Click on Kinetics in the Operations section of the Icon Bar
- a. Choose Steps to Analyze: Association and Dissociation
- b. Choose Model: 1:1
- c. Select Global Fitting (full)
 - i. Group by Sample ID (or other grouping scheme) when performing a parallel experiment
 - ii. Group by Sensor if all concentrations are measured on the same sensor (a serial experiment)
- d. Examine Fitted curves
- e. Steady-State Analysis (Equilibrium Fit)
 - i. Check to include or uncheck to exclude data from the table
 - ii. In Steady-state dialog, choose Response as the mode of analysis (when kinetic data reaches steady-state at each concentration)

- iii. Choose Region of Analysis by defining Average from X to X
 1. This should be a region of the association curve that has reached equilibrium (or steady-state)
 2. Default is a 5 sec window, 5 seconds from the end of the association phase
 3. To view, go to the graph window in the bottom right corner and select the steady-state tab
 - f. Save Excel Report (for a summary of the analysis) or create a custom report
5. Save Data
- a. Go to File Menu
 - b. Click Save or Save As to save processed data in Extended ForteBio File format (*.efrd)
 - c. To reopened saved processed data, click on Open Workspace from the Icon Bar

Last Updated: 2018-09-19

Data Analysis 10.0 (legacy data analysis software)

1. Open the Octet Analysis Software (on the desktop)
2. Go to File→ Load Folder
3. Find Octet Data Folder and click Load to add to the Loaded Data Directory
4. In the Loaded Data Directory, click on experiment name to open it
5. In the Sensor Tray Tab, select the biosensors to be analyzed
6. Go to the **Processing Window**

Processing

STEP 1: Data Selection

1. Click the Sensor Selection Button
2. Assign Parallel Reference Sensors (sensors to which no ligand is immobilized)
 - a. In the Sensor Tray map, the default assignment for all sensors is as Ligand sensors
 - b. Reassign reference sensors by selecting sensors, right-clicking and changing sensor type to Reference Sensor
3. Assign Reference Wells (zero concentration of analyte)
 - a. In the Sample Plate, select the reference wells, right-click and change well type to Reference Well
 - b. Check the box that appeared next to the row with reference wells, to select the reference wells

STEP 2: Subtraction

1. Check the subtraction box (when performing reference subtractions)
2. chose subtraction options:
 - a. Reference Well (subtracts a zero concentration of analyte to correct for drift)
 - b. Parallel Reference Sensor (subtracts a reference sensor to which no ligand has been immobilized, for each concentration of analyte)
 - c. Double Reference (does both Reference Well and Parallel Reference subtraction)

STEP 3: Align Y axis

1. Select Baseline
2. Choose a range for baseline correction (default is the full baseline before association)
 - may choose a truncated region closer to the start of association

STEP 4: Inter-step Correction

- NOTE: for most effective inter-step correction, baseline before association and dissociation steps must be performed from the same well of a sample plate
1. This step corrects for system artifacts (optical artifacts from buffer mismatch, etc.)
 - should not be performed with very fast on-rates
 2. Choose a step to align

STEP 5: Process

3. Select Savitzky-Golay Filtering if measuring protein/protein interactions or when the signal to noise is high. Do not use when signal to noise is low (with small molecule analytes). This removes high frequency noise with a smoothing function.
4. Click Process Data

5. Examine the processed data
6. Save Processed Data and Processing Parameters
7. Go to Analysis Window

Analysis

1. Kinetic Fit
- b. Choose Steps to Analyze: Association and Dissociation
- c. Choose Model: 1:1
- d. Select Global Fitting (full)
 - i. Group by Color, if each concentration is measured on a different sensor (a parallel experiment)
 - *If grouped by color, select rows of data from the table, right-click and change color to a shared color for all data to be grouped*
 - ii. Group by Sensor if all concentrations are measured on the same sensor (a serial experient)
- e. Fit Curves
 1. Steady-State (Equilibrium) Fit
- f. Select rows in table to include
- g. In Steady-state dialog, choose Response as the mode of analysis
- h. Select the Region of Analysis
 - i. This should be a region of the association curve that has reached equilibrium (or steady-state)
 - ii. Default is a 5 sec window, 5 seconds from the end of the association phase
 - iii. To view, go to the graph window in the bottom right corner and select the steady-state tab
 1. Save Analysis Parameters
 2. Save Report (for a summary of the analysis). This does not include kinetic data in a graphable format.
 3. To export data curves:
- i. Right-click on the kinetic curves
- j. Go to the Export Dialog
- k. Choose Text/Data
- l. Choose File
- m. Enter File Name
- n. Click Export
- o. Select Table format
- p. Select Points/Subsets

Last Updated: 2018-09-19