Bio Layer Interferometry (BLI) and Octet Quantitation and Kinetics Assay

Summary

This standard operating procedure (SOP) pertains to processing samples for Biolayer interferometry (BLI) analysis using the Octet® RH16 instrument (Sartorius) and Octet® Discovery software (v13.0).

BLI is a "*label free analysis technique that measures the change in thickness a biolayer (typically due to binding events) on the surface of a sensor*". In a BLI experiment, a fiberoptic biosensor carrying immobilized ligand is dipped into a solution containing a defined concentration of the analyte (**Figure 1A**). The interactions between analyte and ligand molecules result in changes in the interference patterns of white light reflected from the surface of a biosensor tip (**Figure 1B**), which is recorded and displayed in a sensorgram. The main application of BLI is to monitor and quantify biomolecular interactions, allowing for deducing important parameters on kinetics and affinity (i.e. K_D, k_d, k_a).

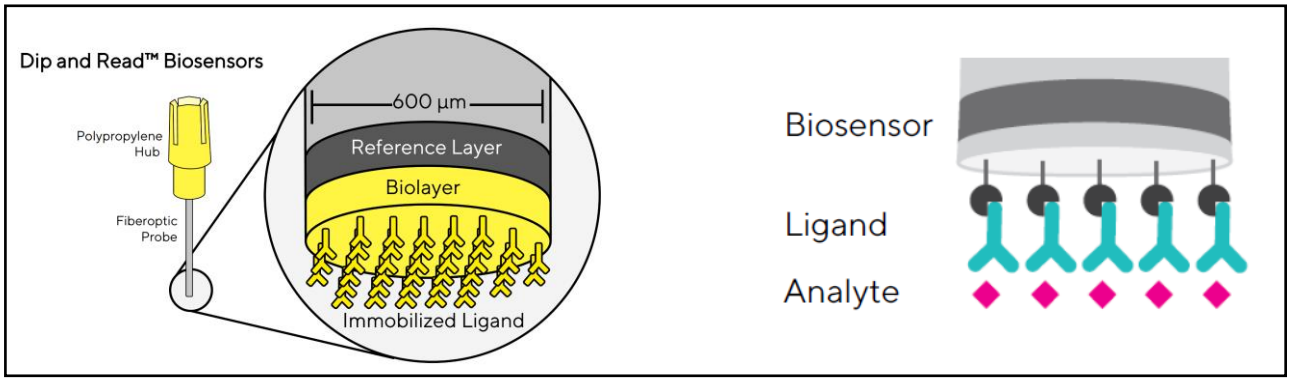


Figure 1A: Biolayer sensor tip

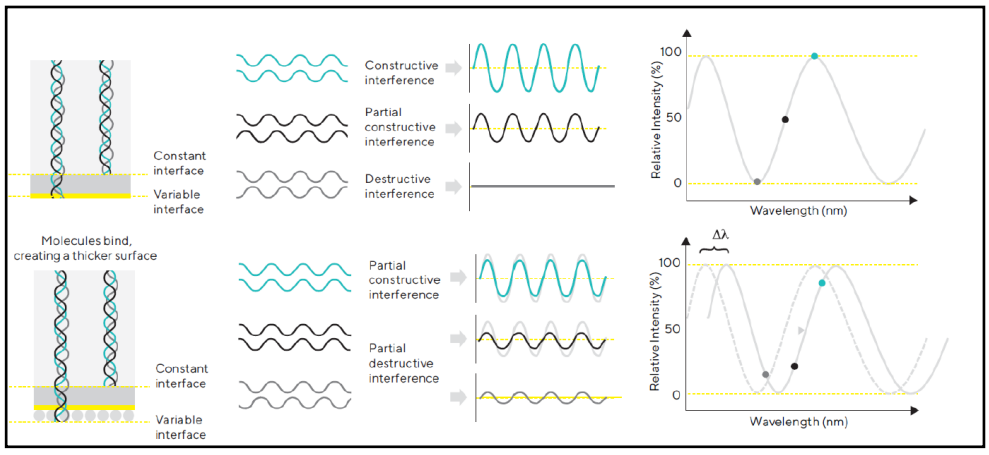


Figure 1B: Principle of biolayer interferometry

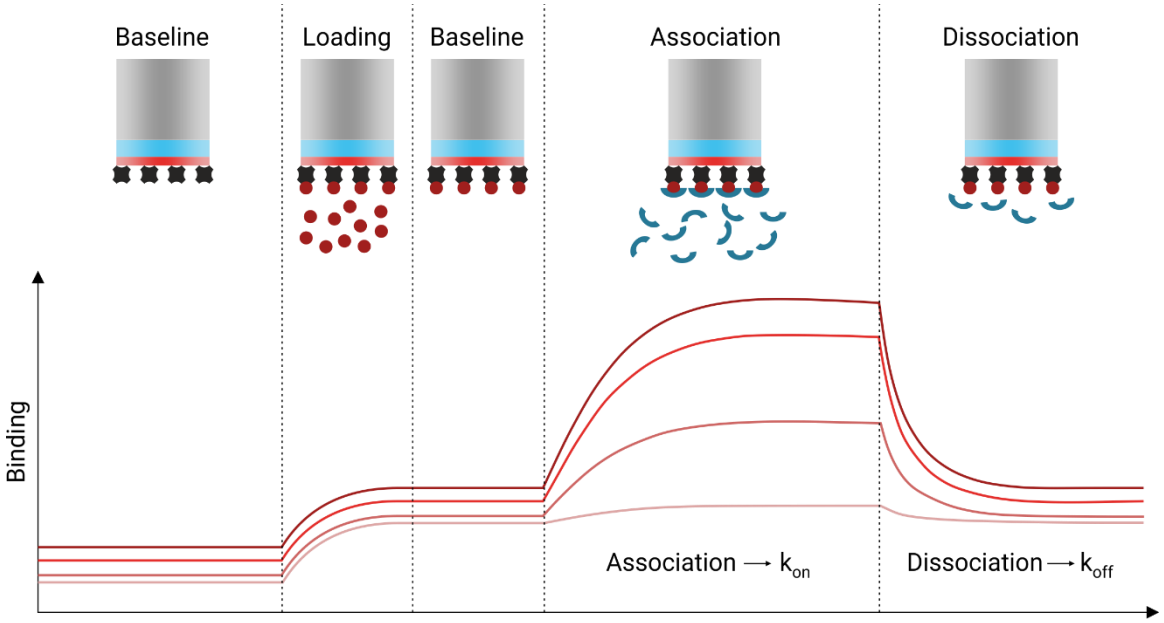
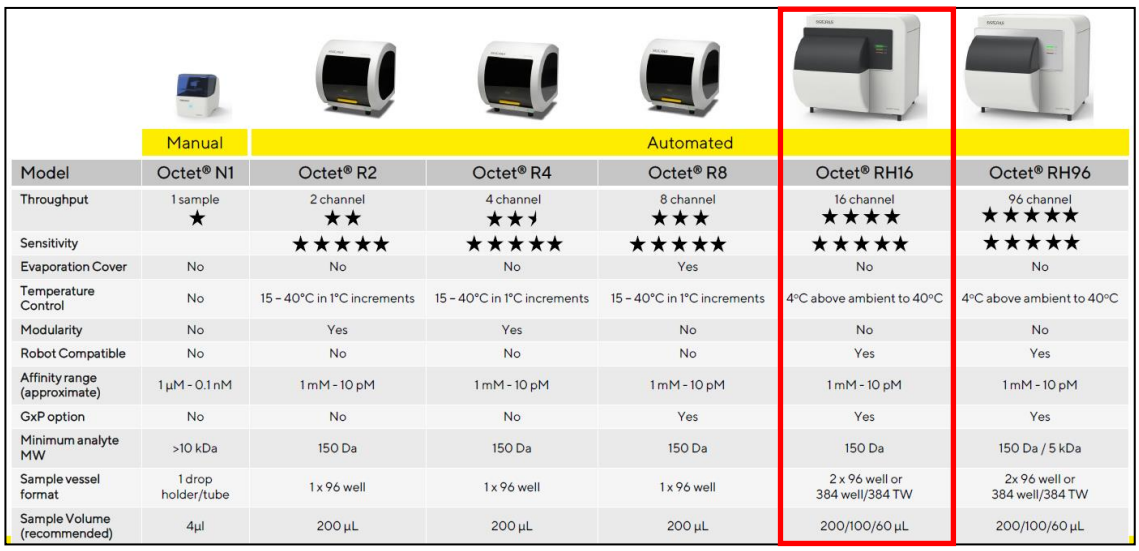


Figure 2: Sensorgram monitoring real-time interactions during a BLI experiment

Instrument (Octet RH16)

The UD facility has an Octet RH16 instrument (Satorius) that offers a high-throughput platform (8 samples/96-well plate or 16 samples/384-well plate) to measure biomolecular interactions. Compared to the lower throughput (i.e. BLItz (>10 kDa)), RH16 offer more advanced features, including an automated platform, higher sensitivity that can detect the signal from analytes that are > 150 Da, wide affinity range (1 mM – 10 pM), temperature control (4-40°C), and low sample consumption (60-200 µL) (**Figure 3**).



	Manual	Automated				
Model	Octet® N1	Octet® R2	Octet® R4	Octet® R8	Octet® RH16	Octet® RH96
Throughput	1 sample ★	2 channel ★★	4 channel ★★½	8 channel ★★★	16 channel ★★★★	96 channel ★★★★★
Sensitivity		★★★★★	★★★★★	★★★★★	★★★★★	★★★★★
Evaporation Cover	No	No	No	Yes	No	No
Temperature Control	No	15 – 40°C in 1°C increments	15 – 40°C in 1°C increments	15 – 40°C in 1°C increments	4°C above ambient to 40°C	4°C above ambient to 40°C
Modularity	No	Yes	Yes	No	No	No
Robot Compatible	No	No	No	No	Yes	Yes
Affinity range (approximate)	1 µM - 0.1 nM	1 mM - 10 pM	1 mM - 10 pM	1 mM - 10 pM	1 mM - 10 pM	1 mM - 10 pM
GxP option	No	No	No	Yes	Yes	Yes
Minimum analyte MW	>10 kDa	150 Da	150 Da	150 Da	150 Da	150 Da / 5 kDa
Sample vessel format	1 drop holder/tube	1 x 96 well	1 x 96 well	1 x 96 well	2 x 96 well or 384 well/384 TW	2x 96 well or 384 well/384 TW
Sample Volume (recommended)	4µl	200 µL	200 µL	200 µL	200/100/60 µL	200/100/60 µL

Figure 3: BLI instrument port

More information on the instruments can be found here.
To operate the instrument, all users **MUST:**

- Carefully review the training materials (PDF files in the Octet training folder on M drive)
- Get trained by either Yanbao or one of the superusers (Ha Le- hale@udel.edu, Megan Doleschal – megdole@udel.edu, Henry Anderson - henrya@udel.edu, Yujue Liu - yujueliu@udel.edu)

Resources:

- **Octet tutorials video series:**
  https://www.youtube.com/playlist?list=PL7wUdfVJXkoDmgZENSjcqOUS3rwrP1Y7p
- **Octet biosensors (second tab on the bottom of the page).** Each biosensor's page has an "additional resource" section that has product data sheet and other useful related information like application and technical notes.

  Octet BLI Biosensors & Kits | Protein Analysis | Sartorius
- **Octet resource library (including application notes => VERY HELPFUL and DETAILED)**
  Octet® Resources | Sartorius

General guidelines:

1. Before you start, consider the following:

- **Which molecule will be immobilized?** The response of BLI correlates with the mass changes at the sensor tips. Therefore, it is recommended to immobilize the smaller molecules on the tips (if possible) and keep the larger molecules as analytes. However, this decision also depends on the availability of each component (i.e. the one of the lower quantity is immobilized even though the other one has a lower molecular weight).
- **Which immobilization method to be used?**
  - Immobilization methods depend on the nature of the biomolecules (i.e. tagged or tag-free, small molecules or large biomolecules, etc.) and will be the determining factor on which biosensor to be used. The biosensor selection guide is here.
  - Direct immobilization is preferred. However, in some cases where direct immobilization is not possible, indirect immobilization might be performed with a high level of control. (i.e. couple the ligand to a linker with a terminal biotin then immobilize the conjugate on a streptavidin biosensor)
- **Which buffer to use?** Components in the buffer can influence biomolecular interactions. Ideal buffer should keep biomolecules stable and functional and not degrade/interfere with the biosensor. Generally, buffers such as PBS or HEPES at pH 7.4 can be used for most systems.
- **How tight is the binding?** The expected/assumed K_D will determine the concentration range of analytes ($0.1K_D$ – $10K_D$).

2. Sample preparation:

- **For biomolecules:**
  - For ligand: since ligand is immobilized on a decorated biosensor tip, it's **VERY IMPORTANT** to have a highly pure ligand, which can be confirmed by a single band on SDS-PAGE gel.
  - For analyte: RH16 is compatible with both crude samples (i.e. serum, lysate, blood plasma, etc.) and pure samples (i.e. purified proteins). However, it is expected that crude analytes will result in a high background and might block/overshadow the interactions of interest. If that's the case, reducing the complexity of the analytes or

blocking the sensor tips is necessary.

- Concentration:
  - Ligand: enough to obtain an increase in signal but not too much that will saturate the biosensor tip
  - Analyte: 0.1 – 10 K_D
- Volume:
  - Require 200 µL for each well of a 96-well plate and 80-120 µL for each well of a 384-well plate

- **Buffer:**
  - As mentioned above, **buffer** composition should **keep the biomolecules stable** and be **compatible with the biosensor.** For example, users should avoid Tris buffer if using amine coupling biosensors for immobilization of compounds with terminal amine. Sartorius sells a universal Kinetics buffer 10X (No. 18-1105) (PBS, 0.02% Tween-20, 0.1% BSA, 0.05% sodium azide)
  - It's recommended that **buffer** should be **filtered** before use.
  - If performing the first experiment, choose the **buffer system in which the biomolecules behave well.**
  - To avoid **signal jumps** between steps, use the **same buffer system** for the whole experiment.
  - Non-specific interactions can cause high background noise and complicate data analysis. It's recommended that **Tween 20 (0.005-0.1%)** (or other detergents in the same class) is added to the buffer system. **BSA** is also recommended when working with proteins.
- **Other supplies**
  - **Plates:**
    - 96-well plates black, flat bottom polypropylene (Greiner Bio One #655209) (Sample volume 180 – 250 µL, recommended 200 µL)
    - 384-well black, flat bottom polypropylene (Greiner Bio One, #781209) (Sample volume 80 -130 µL, recommended 100 µL)
    - 384-well black, tilted bottom polypropylene (Sartorius , #18 5076 or #18 5080) (Sample volume 40 – 80 µL, recommended 60 µL)
  - **Biosensors:**



- Biosensors should be handled with care and protected from light.
- Store at **RT**. **DO NOT** store in **fridge or freezer** or any place that is humid. **Moisture** can **negatively impact the integrity** of the biosensor.
- When transferring biosensors from the original tray to an experiment tray, **use the pipet** that comes with the instrument.
- **AVOID touching** the biosensor!
- **List of biosensors provided by the facility is below (may be updated under users' request):**

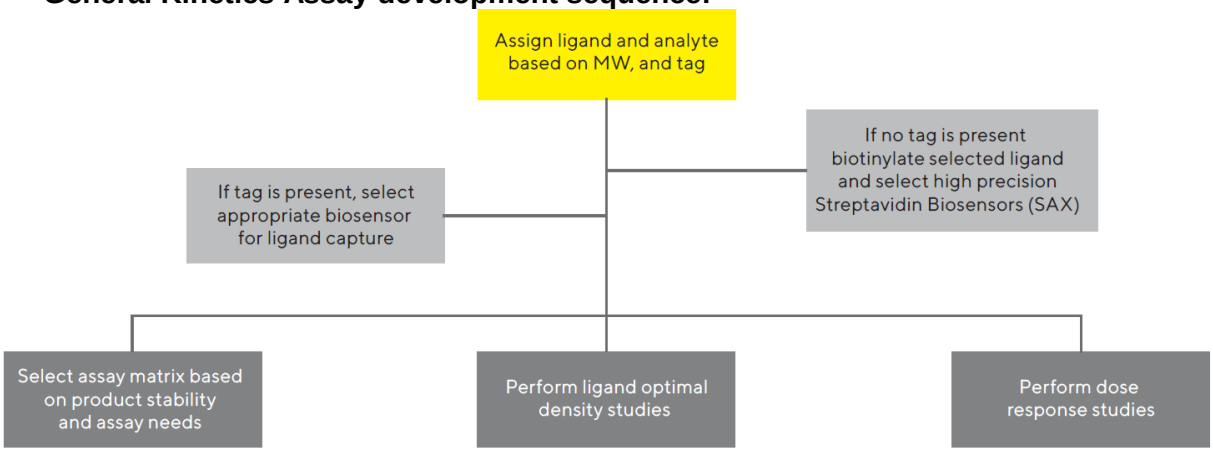
Description	Catalog No.
Anti-Glutathione-S-Transferase (GST)	18-5096
Anti-Penta-HIS (HIS1K) biosensors	18-5120
Streptavidin (SA) Biosensor	18-5019
Protein A (ProA) Biosensors	18-5010

3. Experimental design for Kinetics assay

Ref: "Octet® BLI Kinetics Assay: Method Development Guideline"

If performing Quantitative assay, refer to "Octet® BLI Quantitation Assays: Method Development Guideline" for detailed guideline.

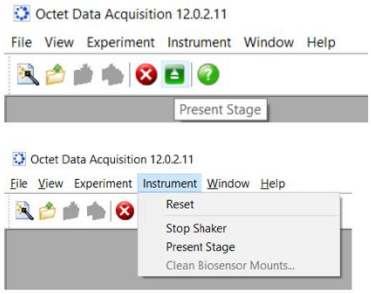
- **General Kinetics Assay development sequence:**



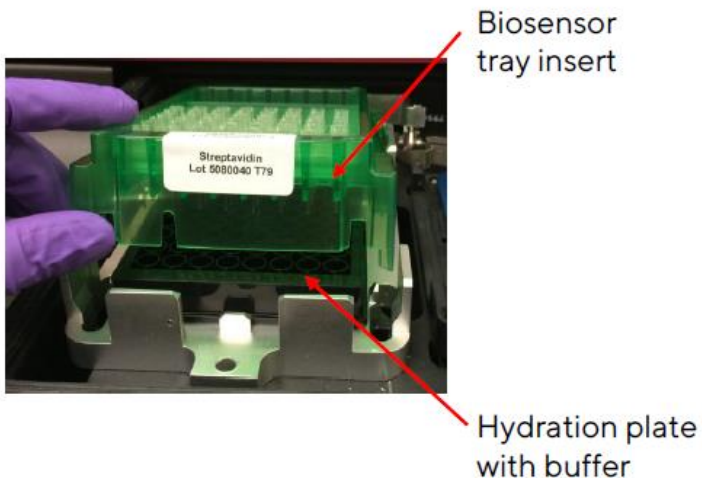
- **Tips on experimental design:**
  - Optimize ligand loading before starting any binding experiment
  - Keep the buffer consistent throughout the experiment
  - Always have controls:
    - **Control for non-specific binding of analytes to the biosensor**: NO ligand and matches the analyte concentration
    - **Control for ligand drifting/dissociation during the experiment:** loaded ligand and buffer without analyte
  - Experiment time: total time should be **<3 hrs** (long experiment time will lead to sample loss due to evaporation

- **Tips on loading optimization:**
  - Over-saturation (too much ligand on a biosensor) => steric hindrance, crowding, possible aggregation, inducing weak non-specific interactions at high concentration of analytes.
  - Too little ligands => signal might be too low for detection
  - **Slow loading with low to medium concentration** of ligands is recommended.
  - **50-100 nM** of ligand is recommended for the loading step
  - A baseline step after "Loading" step should be performed and optimized to minimize ligand drifting (ligand naturally dissociates from the tips)
  - **Ligand density scouting steps are recommended before kinetics experiments**

- **Hydration tips before experiments:**
  - Biosensors **MUST** be **hydrated in the running buffer** before the experiment (~10-20mins)
  - **Hydration protocol:**
    - Transfer biosensors to the experiment rack
    - On the Octet software, choose “Instrument” tab then click on “Present stage”

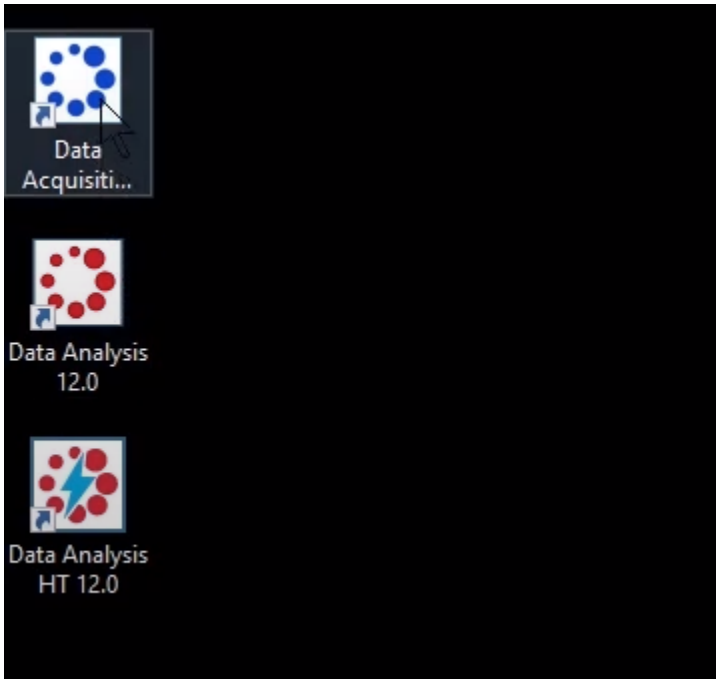


- Pipet 200 µL of running buffer to each well of a 96-well plate using a multi-channel pipet
- Place the hydration plate into the biosensor slot on the left, and place the biosensor tray insert on top. DO NOT PUT THE BLUE TRAY BASE INTO THE OCTET!

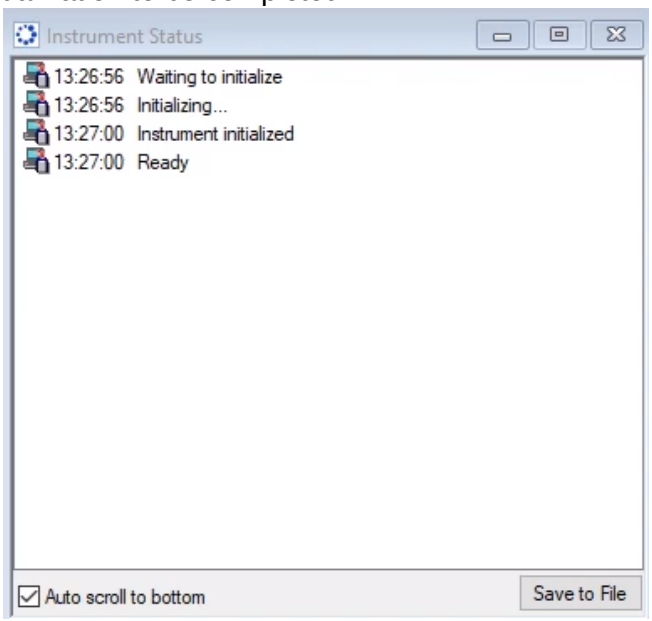


4. Instrument set up

- **Start the instrument**
  - Instrument should be kept “ON” at all times. Otherwise, turn the instrument “ON” and let warm up ~15 mins
  - Turn on the Octet Data Acquisition Software

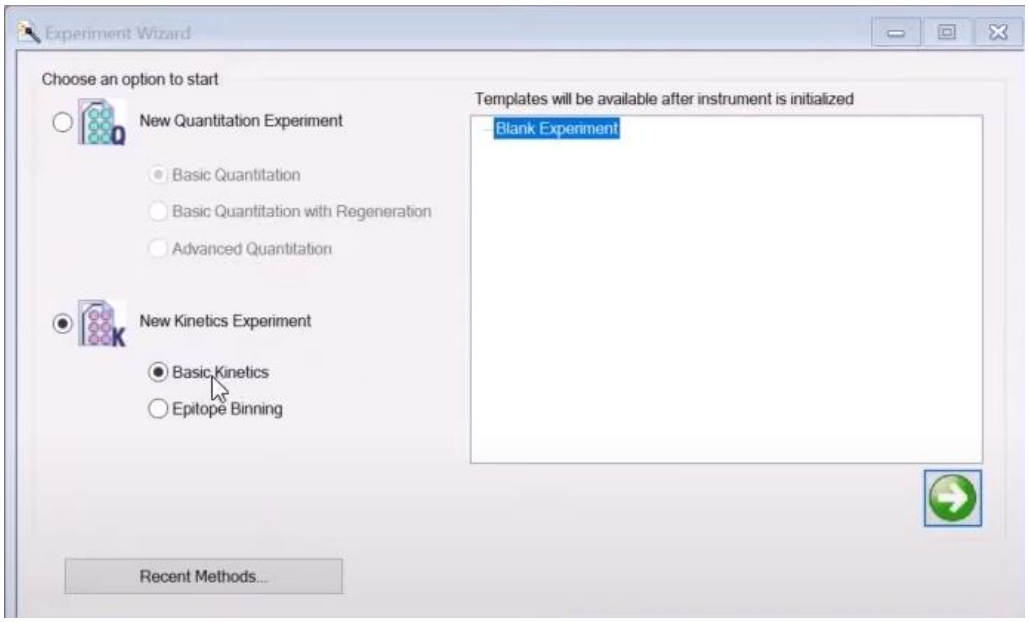


  - Wait for initialization to be completed



  - Select “New Kinetics Experiment” or “Open” → “Method file” (for an existing “Method” file)

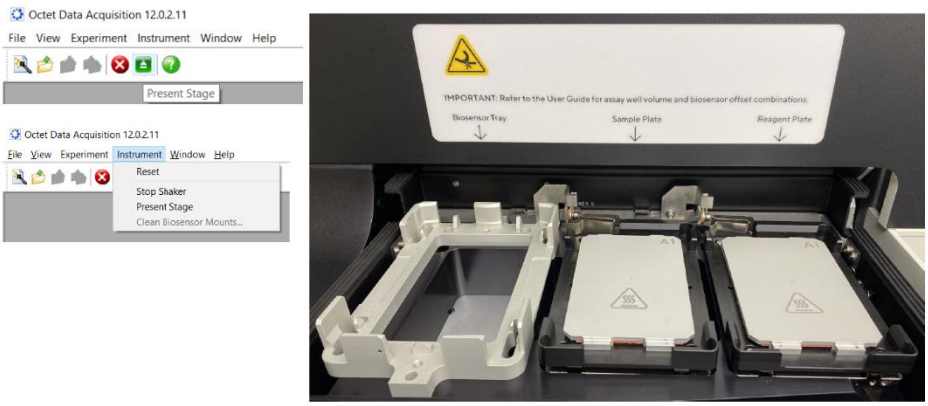
- **Set up a Kinetics experiment (more info on how to set up a Quantitative experiment can be found in the video tutorial series from Satorius)**
    - On the Experimental Wizard box, choose “Basic kinetics” with “Blank template”, click “Go” (the arrow sign)



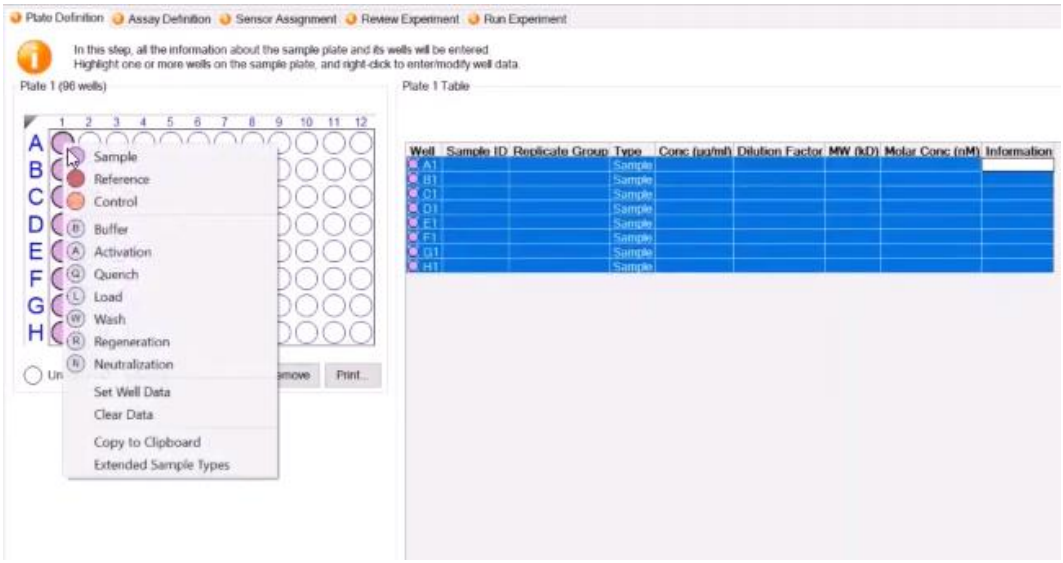
    - At this point, there are 5 tabs corresponding to 5 parameters that need to be changed or modified based on the experiment.

a. Tab1: Plate definition

- In this step, you will assign sample/buffer to each well of the 96/384-well plates
- Choose “Read head” (8 or 16 channels)
    - 8 channels: 1 column of biosensor tips will be picked up at once and will move in 1 column increments
    - 16 channels: 2 columns of biosensor tips will be picked up at once and will move in 2 column increments
- Plate 1: sample plates, Plate 2: buffer plates

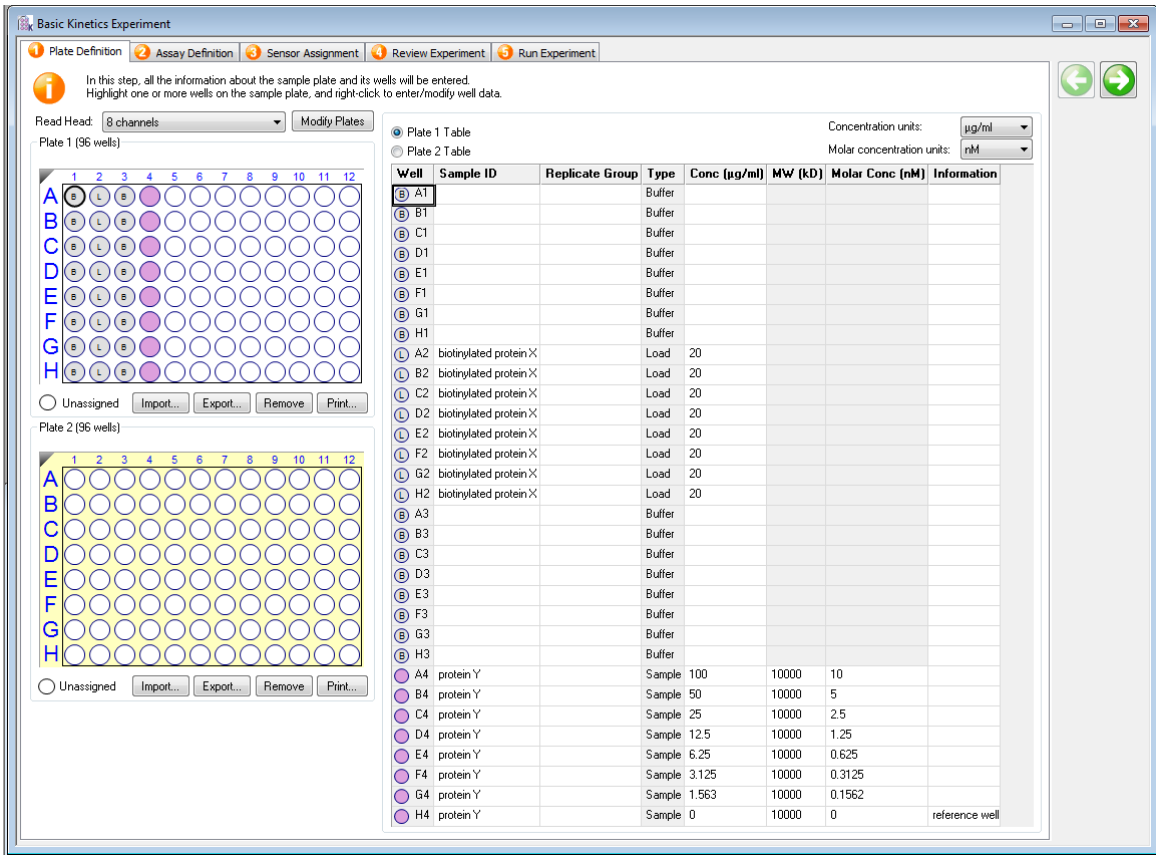


- Modify each column or well by clicking on it and assign the sample type



- The # of columns used is based on the # of steps in the experiment. For example, the steps in exp 1 is: Baseline, Load, Baseline, Association, Dissociation, Baseline, Regeneration. Then, it's recommended to have 7 columns filled out with the corresponding reagents for each step (i.e. running buffer for Baseline, ligand solution for Load, Analyte for Association, running buffer for Dissociation, etc.)

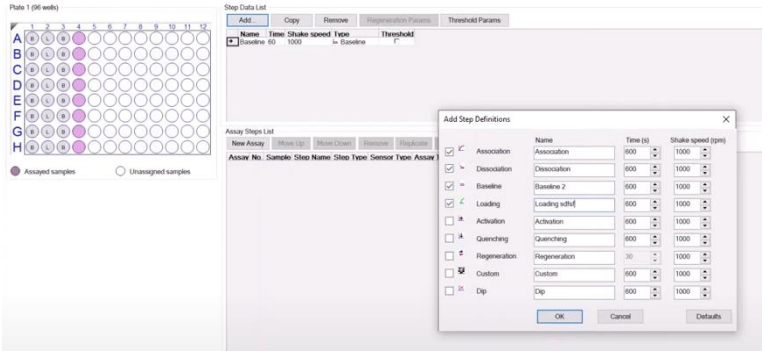
Tab 1. Plate Definition.



- Sample ID, Concentration, MW, Molar Conc (nM) can be individually modified. **Note that Octet software can calculate the molar conc when MW and Conc (ug/mL) of the biomolecules are inserted.**
- **Remember to add at least one reference well**

b. Tab2: Assay definition

- This is where you will set up your experiment (each step of the experiment,

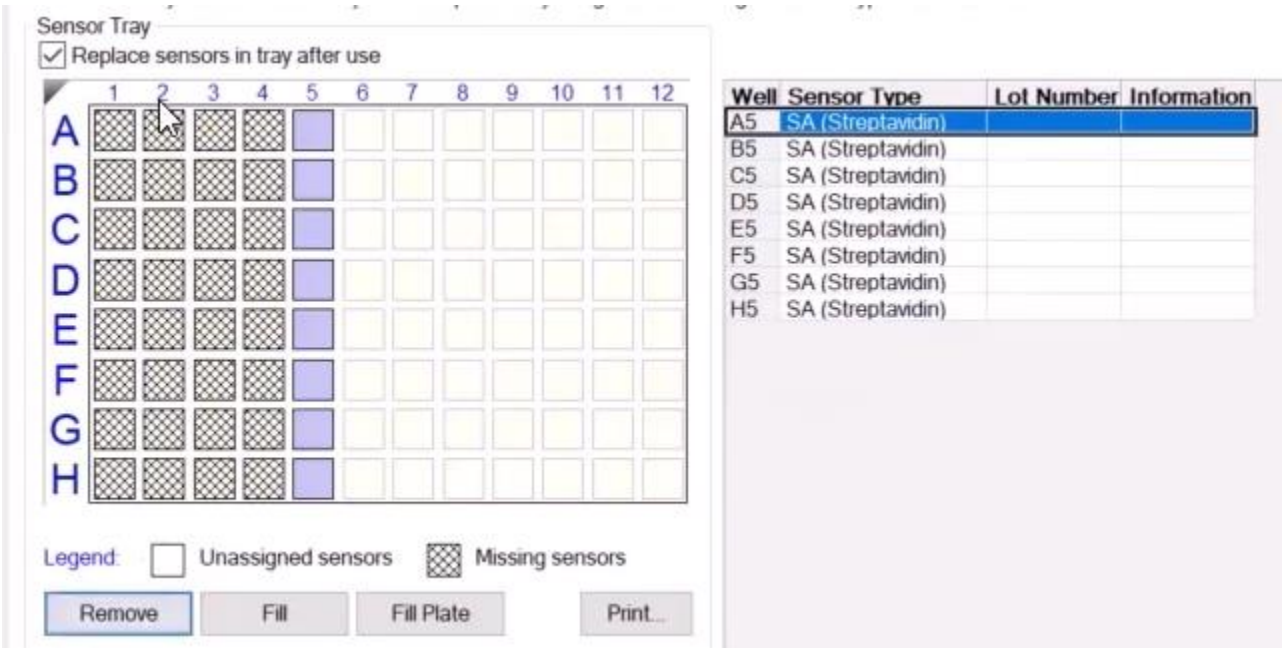


how long each step will take, shaking speed, etc.). Click on “Add” in “Step Data List” to add steps and info

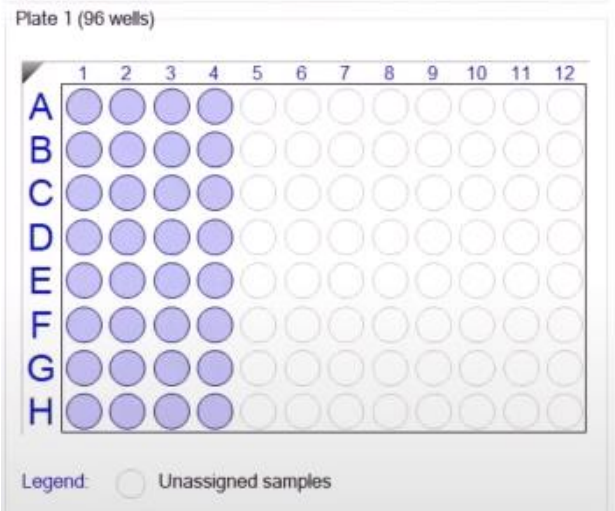
- Recommended 1000 rpm for “Shake Speed”
- In the lower section, choose “New assay” (assign steps to columns of plates)
    - Select a column. Select a step in the “Step Data List”
    - Select the “Sensor type”
- To create a parallel control assay (no ligand and expose the naked biosensors to all concentrations of analytes to account for non-specific binding)
    - Replicate the assay above in the same experiment
    - **Technically, a new experiment can be created but the results of two separate experiments can not be analyzed at the same time (i.e. controls can’t be subtracted from the experiment). It’s recommended to replicate the assay in the same experiment.**
    - For the control assay, change from “Ligands” well to “Buffer well” in the “Load” step. Everything else is the same.

c. Tab1: Sensor Assignments

- In this step, you will choose where to put the sensors in the “Sensor Tray”
- **NOTE: Check “Replace sensors in tray after use” – New sensors will be picked up after each experiment and used sensors will be discarded.**
- The filled squares in the sensor tray mean the sensors in this column will be used for the experiment
- If some sensors are used already and the sensor column starts in the middle, “Remove” the used one → filled square will turn into dashed squares



- Plate 1 (or plate 2) in the lower section shows where in the 96-well plate the sensors will be dipped into

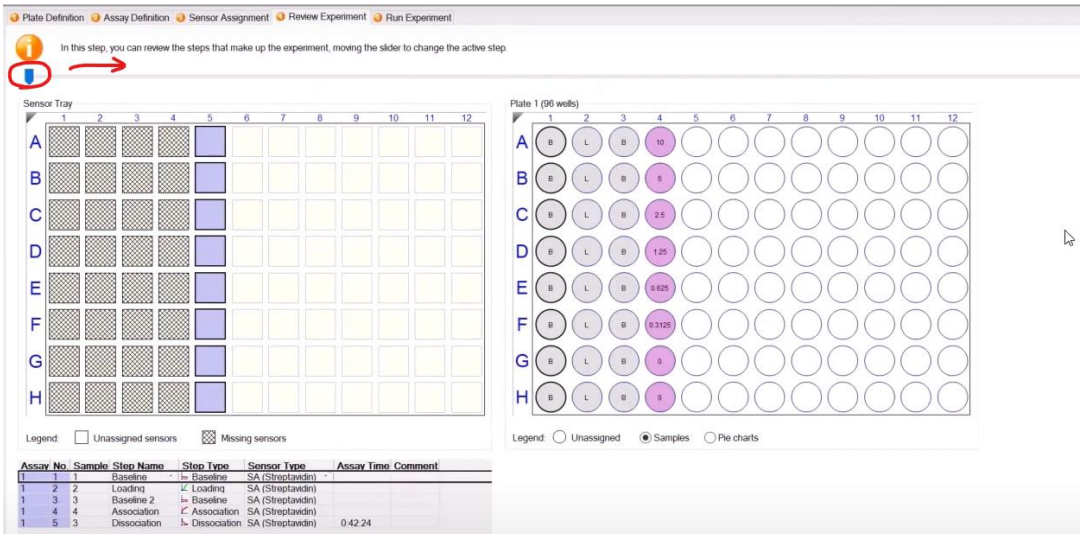


- Sensor type needs to **match** the sensor selection in the "**Assay Definition"** tab

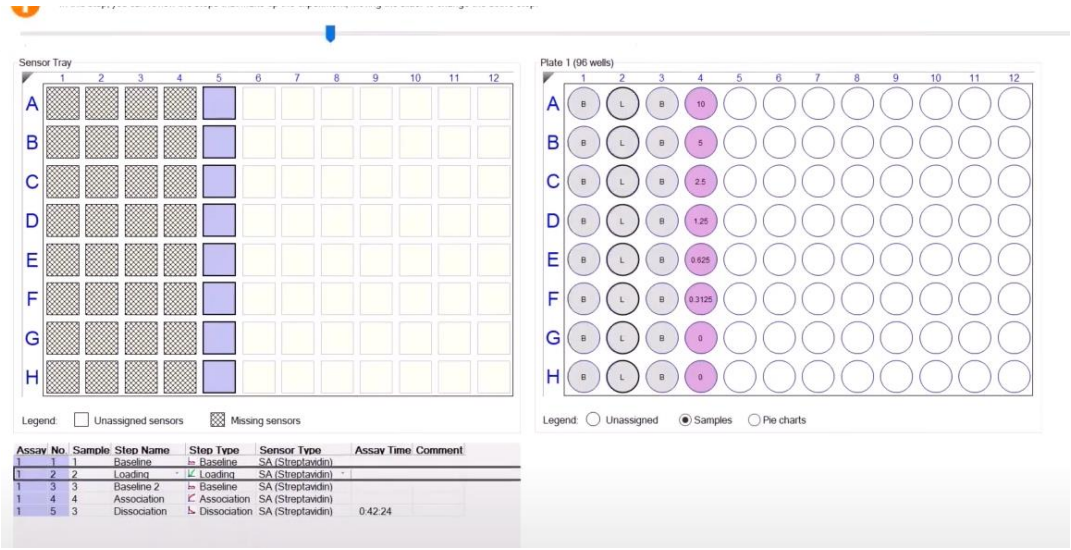
d. Tab4: Review Experiment

- This is where you can review and check for all information before running the experiment.
- Slide the arrow (circled in red in the picture below) to the right to see the simulation of each step in the experiment

Step 1: Baseline



Step 2: Loading



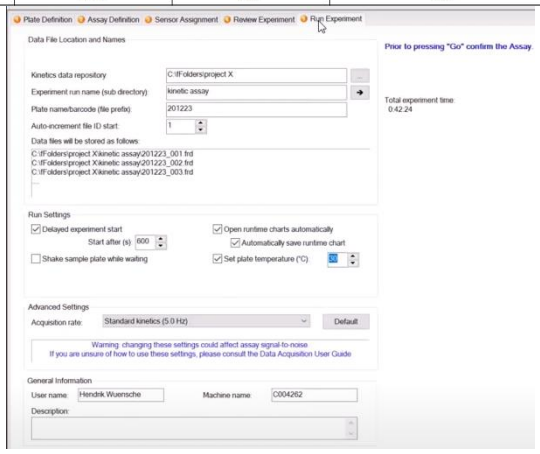
e. Tab5: Run the experiment

- This is where you can choose the destination for acquired data, change the settings, etc.
- In “Kinetics data repository”, select the folder where the data will be saved
- In “Experiment run name”, enter the experiment name (Recommended: [Group]_[Name/Initial]_[date]_[Exp name])
- In “Run settings”:
    - Check all boxes **except “Present stage at end of experiment”** (only check if you will be there when the experiment ends)
    - Temp can be changed based on the experimental conditions. Most of the biomolecular interactions can be monitored at 25°C – 30°C
- In “Advanced Settings”
    - Don’t change “Sensor Offset (mm)” unless the sample/reagent volume is changed

      Recommended Sensor Offset is below:

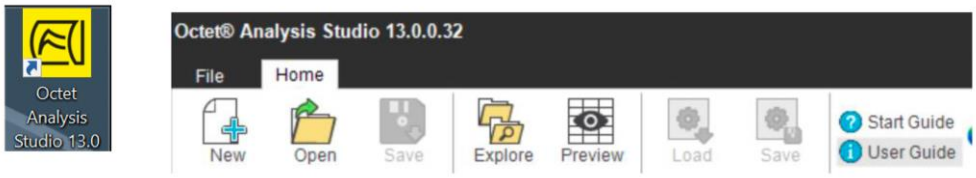
Table 2.3 Sensor offset and well volumes for Octet RED384 & QK384

Sensor Offset (mm)	Recommended Minimum Fill Volume (µl)		
	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

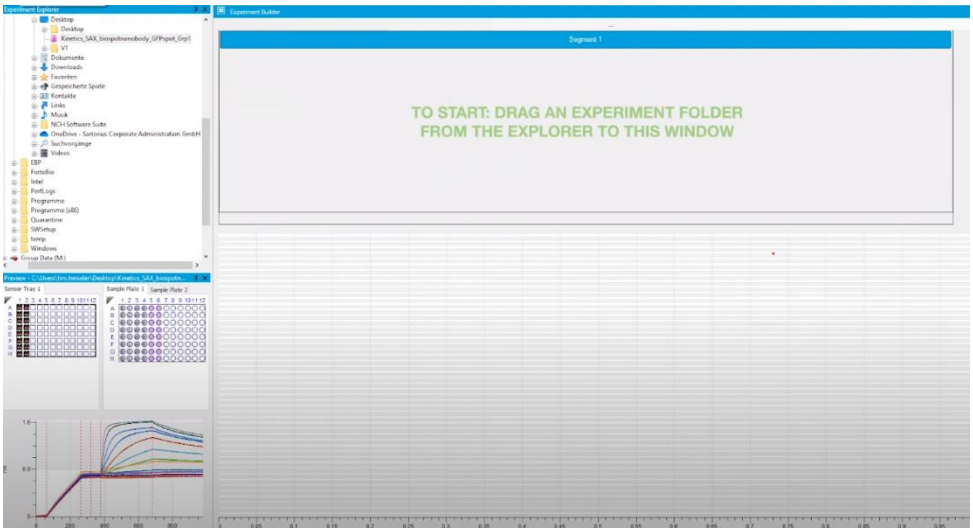


  - Set Acquisition Rate as recommended
- Save the method if want to use it later
- Click on “Go” button to start the experiment

- **Data analysis**
  - Open “Octet Data analysis” software

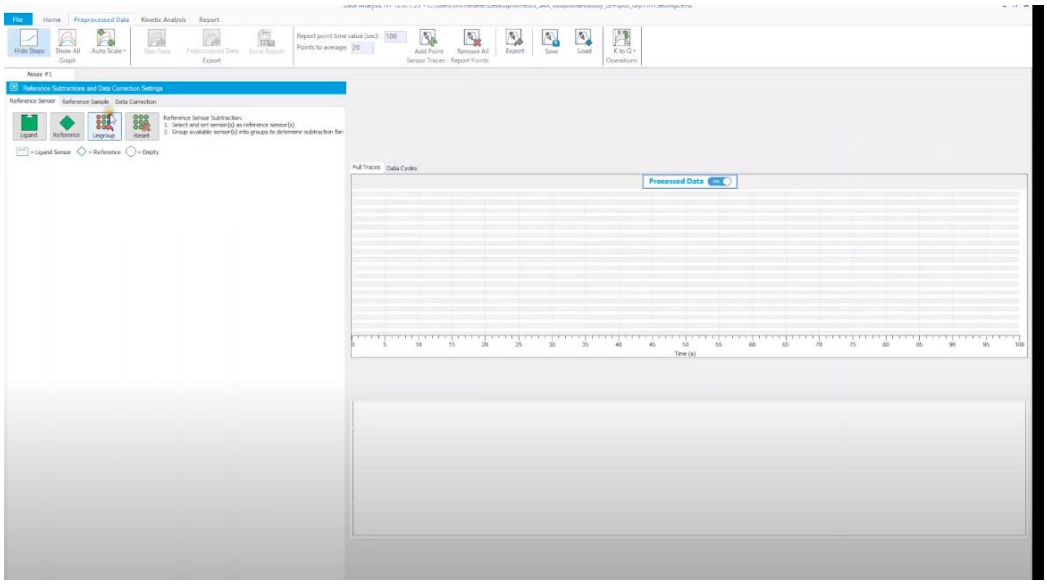


  - Select data from the data folders
    - More than one file can be loaded by dragging the additional files into the “Overlay” section



a. PreProcess data tab

- Here, the reference signals will be subtracted from the experimental signals



- Subtract the signals from **reference sensors** (sensor without ligand that was dipped into analyte solutions):
    - Assign reference sensors: Choose the sensor, right click, then choose “Reference sensor”

    - Choose the experimental sensors then right click, choose “Subtract reference” and choose the subtraction method (i.e. “In rows”, etc.)
- Subtract the signals from **reference samples** (samples without analytes, accounting for ligand drifting):
    - Assign reference samples: Choose the sample, right click, then choose “Reference sample”
    - Choose the experimental samples then right click, choose “Subtract reference” and choose the subtraction method.

b. Data Corrections:

- This is the step where you can align different curve so every curve has the same starting point, accounting for mismatch in signals between different sensors
- “Align Y axis”: average the baseline step
- “Inter step correction”: correct for mismatch in buffer
    - Either association or dissociation steps

c. Kinetics analysis

- This step is to fit the data to binding models

- **Fitting Parameters:**
  - Click on Kinetics tab
  - Choose steps to analyze:
    - Both Association and Dissociation
    - Association (ka, equilibrium)
    - Dissociation (kd)
  - Choose model: 1:1 **(only choose other options if you know for sure your system is not 1:1 binding)**
  - Fitting type (mostly Global fitting)
- Can remove outlier concentration to improve fitting
- **Check for quality of fitting**
  - Check R^2 and X^2 value to see if the fitting has been improved
  - Check residues graph: make sure signals are <10% of signal
- Save Excel report