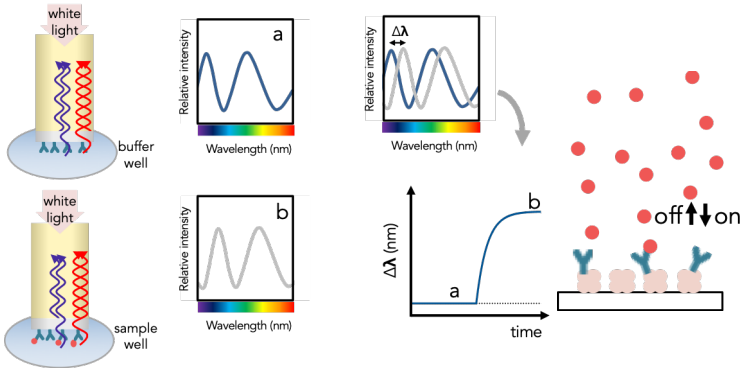
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# Octet: CMI Getting Started Guide to Biolayer Interferometry for Binding Kinetics

Sample Preparation | Getting Started | Data Collection | Data Analysis | Shutdown | Data Management

## Introduction

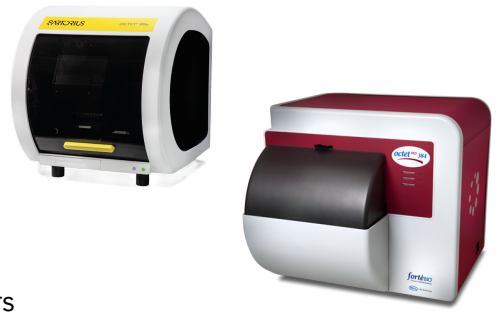
Biolayer Interferometry (BLI) is an optical technique that measures macromolecular interactions by analyzing interference patterns of white light reflected from the surface of a biosensor tip. BLI experiments are used to determine the kinetics and affinity of molecular interactions. In a BLI kinetic experiment, the Octet biosensors dip sequentially into wells of a microplate for each step of an experiment. First, one molecule (the **Load Sample**) is immobilized and then binding of a second molecule (the **Analyte Sample**) is then 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.




| | Octet R8e | Octet Red384 (RH16) |
|---|---|---|
| Spectrometers | 8 | 16 (2x8) |
| Reagent plates | 1 plate<br>384 or 96w | 2 plates<br>384 or 96w |
| Sample Volume | 60– 200 µL | 60– 200 µL |
| Temperature Control | 15 – 40 C | 30 – 40 C |
| Sensitivity | Higher | High |
| Acquisition rate | 5 Hz<br>(up to 40 Hz) | 5 Hz<br>(up to 10 Hz) |
| Run time | Up to 3 hours<br>Up to 16 hours (w/evaporation cover) | |

## Instrument Overview

The CMI has **Octet R8e** and **Octet RED384 (now called Octet RH16)** instruments from Sartorius (formerly ForteBio). The Red384 has reached end of service and will be available for as long as parts are available. The R8e has enhanced sensitivity with good signal to noise at lower Rmax and improved detection of small molecules. Using Octet biosensors and black microplates, these instruments measure Biolayer Interferometry (BLI) for binding kinetics, binding equilibrium and for protein quantitation. The Octet Data Acquisition software is called **Octet BLI Discovery** and the Octet Data Analysis software is called **Octet Analysis Studio**.



### *Applications*

- Kinetic binding: $k_a$, $k_d$
- Equilibrium binding: $K_D$
- Macromolecular and small molecule binding

### *Key Features*

- Disposable biosensors (regeneration not required)
- Simultaneous real-time binding on 8 or 16 biosensors
- 96 or 384 well reagent plate format

*Required Supplies*

- Sartorius BLI Biosensors.
    - See table below for popular sensor types and part numbers. Go to the Sartorius BLI website (formerly ForteBio) website for additional sensor types.
- At least two black microplates per experiment
    - Black 96-well plate for hydrating sensors
    - Black 96 or 384-well plate for samples and reagents
    - Only **Greiner Bio-One brand, black microplates** or **Octet plates** are recommended (see table below).
- An empty biosensor tray to use as a working tray (available at CMI).
- Pipettes (recommended).

| **Select Octet Biosensors for Kinetics** | **Sartorius Part Number** |
|---|---|
| Streptavidin (SA) biosensors | 18-5019 (96/tray) |
| High Precision Streptavidin (SAX) biosensors | 18-5117 |
| Super-Streptavidin (SSA) biosensors (for small molecules) | 18-5057 |
| anti-His (HIS1K) biosensors | 18-5120 |
| Ni-NTA (NTA) biosensors | 18-5101 |
| Anti-Human IgG Fc (AHC) biosensors | 18-5060 |
| Anti-Mouse IgG Fc (AMC) biosensors | 18-5088 |
| Anti-Flag (DYKDDDDK) Biosensors | 18-5187 |
| **Octet Black Microplates** | **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 µL | 781209 (VWR 82051-318) |
| Sartorius Octet 384TW microplates, tilted-bottom, 60 µL | 18-5166 |
| Sartorius Octet Evaporation Covers | 18-5132 |

## Sample Preparation

*Assay Buffers*

- Many buffers are compatible with BLI. It's usually a good idea to start with a buffer system in which your proteins are well behaved.
- **Addition of 0.05% Tween 20 (or other blocking agent) is usually required** to prevent non-specific binding, which is a frequent problem in BLI experiments.
    - Try detergent concentrations above the CMC, typically in the range of 0.02-0.1%.
    - 0.1% BSA may also be used (typically in addition to detergent). BSA is not universally beneficial and may increase non-specific binding and may suffer from batch effects.
- The sample used for the association phase should be in a buffer identically matched to that used for the baseline and dissociation phase.
    - Buffer match is especially important when a buffer component has a high refractive index, such as DMSO. Immobilized load sample should also be in the same buffer, if possible.

*Hydration Buffer*

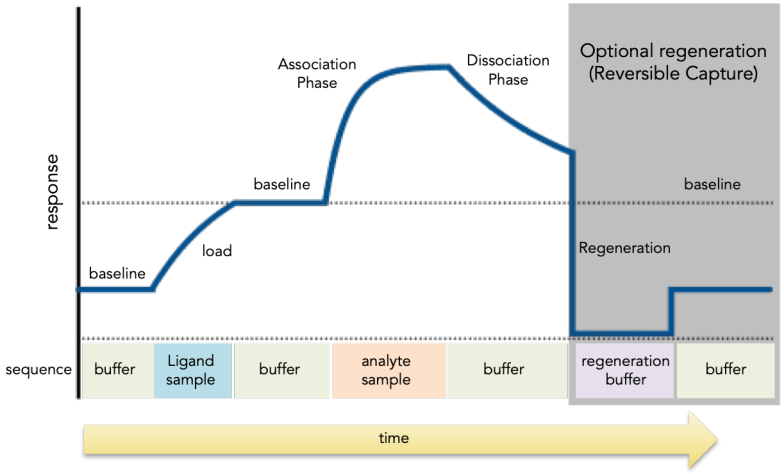
- Sensors must be rehydrated (soaked) before use for minimum of 10 minutes
- Hydration buffer is typically either your assay buffer or your assay buffer without any blocking agents (such as Tween 20 or BSA).

*Samples*

- All BLI experiments are setup with one molecule fixed to the biosensor surface (the Load Sample) and a second molecule in solution (the Analyte Sample).
- Concentration should be accurately measured
    - Errors in Load concentration can affect signal intensity
    - Errors in the Analyte concentration will directly translate to errors in the $K_D$
- Protein aggregates will interfere with BLI.
    - Filter or centrifuge samples before use.
    - Assess protein heterogeneity via light scattering.
    - Purify protein samples with soluble aggregates by size-exclusion chromatography.
- Recommended initial concentration ranges:
    - Analyte 0.1 – 10 X $K_D$ (100 X $K_D$ for high affinity interactions)
    - Load Sample (immobilized) 10-50 µg/ml (µM or lower)
        - Adjust load concentration and/or duration to get the lowest acceptable analyte Rmax. A linear load phase minimizes the chance of overloading.
        - Target Rmax values: Red384 Rmax ~ 0.2 – 0.5 nm
          R8e Rmax ~ 0.1 – 0.2 nm
- Sample and Reagent plate well volume
    - 96-well 200 µl
    - 384-well 80 µl
    - 384-well tilted bottom 60 µl

## Getting Started

An Octet experiment involves multiple steps in which BLI Biosensor are moved between wells in microplates containing buffers, reagents and samples. The instrument can hold up to 96 hydrated biosensors (one tray) for use in experiments with multiple assays. Reagents and samples are placed in 96- or 384-well black microplates. Plate 1 is used on both instruments and in all experiments and can hold any/all sample or reagent types. On the Red384, an optional Plate 2 cannot hold analytes but can hold other reagents.




*Resources*

Additional resources are available at the instrument, including Data Collection and Data Analysis software manuals.

*Experimental Design Tips*

- Do not overload the immobilized molecule.
- The same well containing buffer should be used for the baseline and dissociation phase, assuming inter-step correction is performed.
- For small molecule work, use Super-Streptavidin sensors and quench with biocytin (biotinyl-lysine at 10 µg/mL).
- Use reference subtraction (there are several types).
    - ***Reference sample well*** has immobilized load sample and no analyte during association.
    - ***Reference sensor*** is a sensor to which *no load sample* is immobilized and is matched for analyte concentration.
    - ***Double Reference*** uses both reference well and reference sensor.
- Experiments without an evaporation cover should be less than 3 hr (10 % volume loss ~ 3.5 hours at 30 ˚C).

## Data Collection

*Startup*

1. Book time on the Octet Red384 or R8e PPMS calendar before you start.
2. Login to the computer using your PPMS credentials (eCommons ID and password).
3. The instrument should be powered on at all times.
    a. To restart or cycle power, the Red384 power supply is on the shelf above the instrument, and the R8e power button is on the back right side.
4. Start the Octet BLI Discovery Software. The current version will be on the Desktop.
    a. DO NOT open the door during initialization or you'll have to restart the software.
    b. Wait for initialization, until Instrument Status: Ready.
5. Open door:
    a. Red384: Open the instrument door with the *Present Stage* button in the software (eject button).
    b. R8e: Open the instrument door manually by lifting the handle.

*Experimental Data Collection*

1. Start the Octet BLI Discovery Software. The current version will be on the Desktop.
    a. Wait for initialization.
    b. Select New Kinetics Experiment in the Experiment Wizard or open a method file.
    c. The Experiment Wizard has 5 sections tabs to guide you through experimental setup.
2. Plate Definition:
    a. Red384 only: Select data acquisition mode. Read Head: 16 channel or 8 channel.
        i) 16 channel mode uses up to 16 sensors and moves in 2 column increments.
        ii) 8 channel mode uses up to 8 sensors and can move in 1 column increments.
    b. R8e or Red384: To modify plate format, select Modify Plate:
        i) Choose either 96-well or 384-well format for plate 1 (and/or plate 2 on Red384)
    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) Required Reagent Types
(1) Buffer (baseline before load)
(2) Load Sample (load)
(3) Mock Load Buffer (for reference sensor load step)
(4) Buffer (baseline before association and dissociation)
(5) Sample (association phase), use a range of concentrations, include 0 for reference sample
iv) Optional Reagent Types
(1) Quench (e.g. biocytin for blocking)
(2) Regeneration solutions (for sensor reuse or serial data collection)
v) Always include a zero concentration of analyte (a reference sample well) to correct for a drift in the baseline. Several sensor types have significant drift.
vi) Test non-specific binding with Reference Sensors (sensors with no load, measured with each concentration of analyte.)

e. Fill your reagent plate(s) according to the plate map.

3. 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):

| | | | |
|---|---|---|---|
| (1) Baseline | 120 | (60 – 300) | |
| (2) Loading | 120 | (120 – 600) | |
| (3) (Quench) | 60 | (30 – 120) | optional quench (eg. Biocytin on SA or SSA) |
| (4) Association | 300 | (60 – 600) | |
| (5) Dissociation | 600 | (60 – 600+) | |

ii) Shake speed 1,000 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) Typical assay order:
(1) Baseline
(2) Loading
(3) Baseline
(4) Association
(5) Dissociation

A group of "Baseline – Association – Dissociation" steps are required for recognition as a binding assay and can be overlayed for kinetic analysis. Don't forget the baseline before association.

d. To create new Assays, click New Assay or select all steps in Assay 1 and click replicate. Modify the sample column as needed.

4. 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).

   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 a 96-well plate underneath with hydration buffer in the wells with sensors.

5. Review and prepare Experiment:
   a. Click the arrow to review each assay step in your experiment.
   b. Set up your hydration plate and reagent plate(s) according to your plate maps.
   c. Use the same volume for each well on your plate(s):
      i) 96-well plate 200 µl
      ii) 384-well plate 80 µl
      iii) 384-well tilted bottom 60 µl
6. Run Experiment:
   a. Assign a location for your data (choose your folder **inside your lab folder**).
   b. Enter the experiment run name (avoid using the default name Experiment_1).
   c. **Red384 Run Settings:**
      i) Set experiment delay for hydration and equilibration time (10 min).
      ii) Uncheck "Present Stage at end of experiment" to prevent door from at the end of your experiment.
      iii) Set temperature 30 C (minimum 30 C to 40 C).
      iv) Advanced Settings:
         (1) Set Sensor Offset: 4 mm for standard well volumes defined above.
         (2) Set Acquisition Rate: 5Hz default (1 – 10 Hz).
   d. **R8e Run Settings:**
      i) Set experiment delay for hydration and equilibration time (10 min).
      ii) Set temperature 30 C (minimum 15 C to 40 C).
      iii) Advanced Settings:
         (1) Set Sensor Offset: 4 mm for standard well volumes defined above.
         (2) Set Acquisition Rate: 5Hz default (1 – 40 Hz).
   e. Save method file if using later.
   f. Start Run by pressing Go button.
7. During Run:
   a. Watch data collection in real-time.
   b. Avoid skipping or extending steps, especially if performing an experiment with a second assay (such as a reference assay). Only assays with identical number of steps and duration of steps can be merged for subtractions or comparison.
   c. When data collection is complete, close the Data Acquisition software and go to Data Analysis.
   d. Do not open the door on R8e during data collection (when yellow light is on) as this will abort experiment.

## Data Analysis

*Data Analysis Studio features:*

- Data from multiple plates and/or experiments can be combined into one analysis.
- Flexible reference subtraction options are available for kinetic analysis.
- Customizable report format.
- Export Results as a single file.

*Data Analysis Protocols*

1. Open the Octet Analysis Studio 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.
         (1) To combine data with different step sizes.
         (2) 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 the Reference Sensor Tab.
      ii) Click on column number or drag to select the 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.
         (1) In Rows (selects a reference from the same row as the sample).
      vii) Scroll down to see multiple plates and repeat the subtraction 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.
      vii) In columns (selects a reference well from the same column as the samples).
      viii) Scroll down to see multiple plates and repeat for each plate.
      ix) Check the table to see the well subtraction formula for each sensor.
   d. Data Corrections:
      i) Go to Data Correction Tab.

ii) Align the Y axis to the average of baseline step (default is the last 5 seconds of the baseline).
iii) Inter-step correction.
(1) This step corrects for system artifacts (optical artifacts from buffer mismatch, etc.).
(2) Choose a step to align (try dissociation first).
(3) The baseline before association and dissociation steps must be performed from the same well of a sample plate.
(4) Should not be performed with very fast on-rates as kinetic data may be lost.
iv) Noise Filtering (Savitsky-Golay Filtering, smoothing function) is recommended but optional.

e. Export Processed data by clicking 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:
i) Click on Kinetics in the Operations section of the Icon Bar.
ii) Choose Steps to Analyze: Association and Dissociation.
iii) Choose Model: 1:1.
iv) Select Global Fitting (full).
v) Group by Sample ID (or other grouping scheme) when performing a parallel experiment.
vi) Group by Sensor if all concentrations are measured on the same sensor (a serial experiment).
b. Examine Fitted curves.
c. 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 (time interval).
(1) This should be a region of the association curve that has reached equilibrium (or steady-state).
(2) Default is a five sec window, five seconds from the end of the association phase.
(3) For viewing, go to the graph window in the bottom right corner and select the steady-state tab.
d. Save the Excel Report (for a summary of the analysis) or create a custom report
e. Export Results as a single file or multiple files for re-graphing and/or analysis in 3rd party software.
f. Processing parameters are autosaved in the HTSettings.efrd
i) To restart with default/no settings, you may delete the HTSettings file and reopen.
ii) Save As to save processing parameters in Extended Octet File format (*.efrd).
iii) Click on Open Workspace from the Icon Bar to reopen saved processed data.

## Shutdown

1. Remove sensor tray and reagent plates from the instrument.
2. Close the Octet door.
3. Discard used biosensors with tips.
4. Return borrowed empty sensor trays to the drawer under the instrument.
5. Clean up in and around the instrument.
6. Close the control and analysis software.
7. Logoff from PPMS!

## Data Management

| | |
|---|---|
| Technology | Biolayer Interferometry |
| Instruments | Octet RH16 (Octet Red 384), Octet R8e |
| Recommended Repository | Generalist Repository |

### Data Collection Software

| | | | |
|---|---|---|---|
| Current Version | Octet BLI Discovery, Version 13.0 Red384, Version 13.1 R8e | | |
| Data Files (Type, ~size) | experiment folder (contents below) | | 5 MB/experiment |
| | data file | .frd | 80 KB/measurement |
| | method file | .fmf | 35-45 KB/experiment |
| | plate definitions | .jpg | 30-90 KB/plate |
| | assay image | .jpg | 30-90 KB/experiment |
| | preprocessed data | .xslx | 200 KB/experiment |

### Data Analysis Software

| | | | |
|---|---|---|---|
| Current Version | Octet Analysis Studio, Version 13.0 Red384, Version 13.1 R8e | | |
| Data Files (Type, ~size) | HT Settings | | |
| Readable Exports | results table | .xslx | 5-10 KB |
| | export report | .xslx | |

Book time and Report Problems through the PPMS system: https://ppms.us/hms-cmi

- rates are based on *booked and real time usage*

Contact cmi@hms.harvard.edu with questions.

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