

Octet RED385: CMI Getting Started Guide to Biolayer Interferometry

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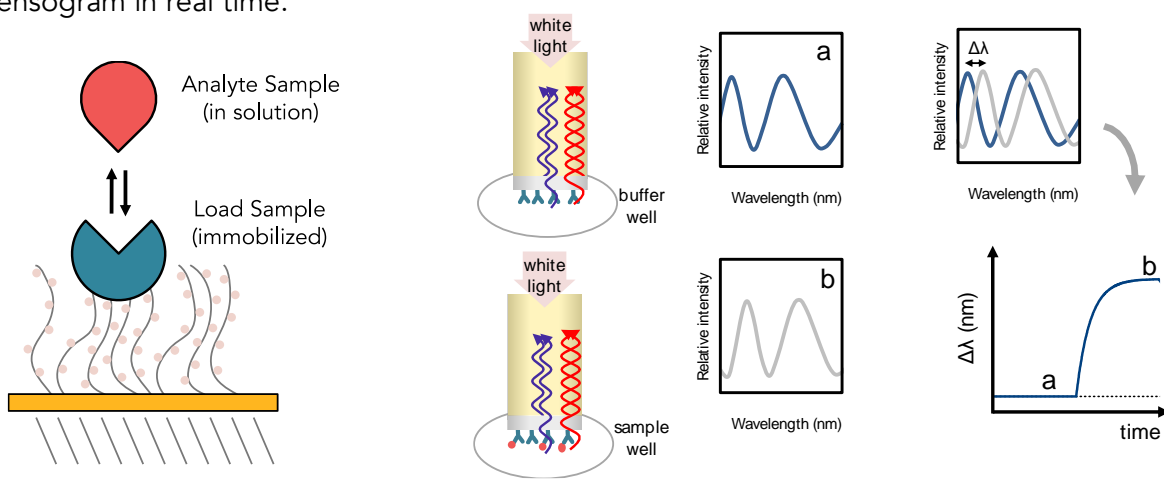
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Introduction

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 experiment, one molecule (the **Load Sample**) is immobilized to a Dip and Read Biosensor and 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. Response is recorded and displayed on a sensogram in real time.



Instrument Overview

The CMI has an **Octet RED384** instrument from ForteBio, which measures binding kinetics and equilibrium by BLI using ForteBio dip and read biosensors and black microplates.

Applications

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

Key Features

- Simultaneous real-time binding on up to 16 biosensors
- Disposable biosensors (sensor regeneration not required)
- 96 or 384 well reagent plate format
- Small molecule sensitivity

Required Supplies

- ForteBio Biosensors.
 - See table below for popular sensor types and part numbers. Go to the ForteBio website: <http://www.fortebio.com/biosensor-types.html>, for additional sensor types, including Anti-Mouse IgG Fc, Anti-Human Fab, Anti-GST, and biosensors recommended for quantitation.
- At least two black microplates per experiment (one for soaking sensors and at least one for samples and reagents).
 - Only **Greiner Bio-One brand, black microplates** or **ForteBio plates** are recommended (see table below).
- An empty biosensor tray to use as a working tray.
- Pipettes (recommended).

Popular ForteBio Dip and Read Biosensors for Kinetics	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 biosensors	18-5010

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

Sample Preparation

Assay Buffers

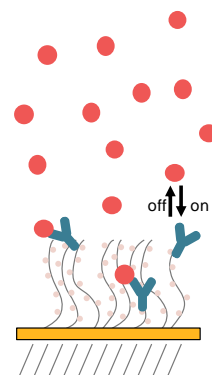
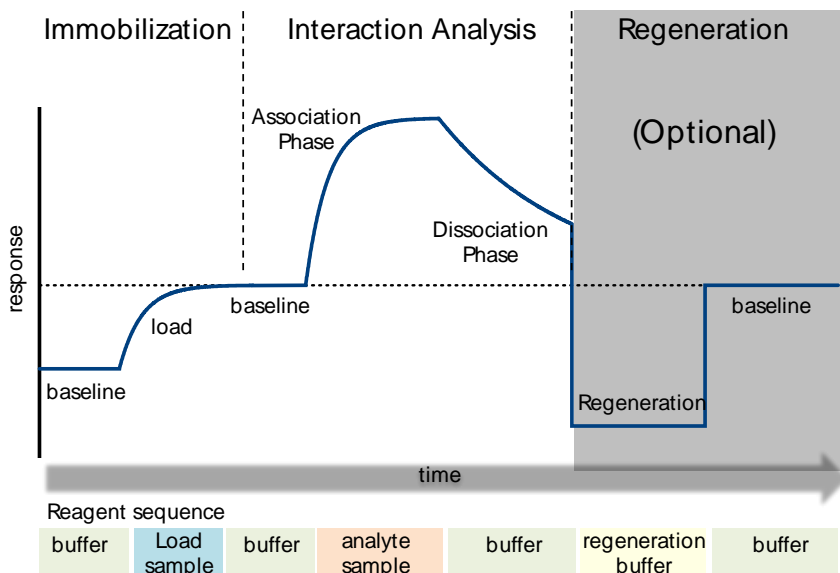
- Many buffers are compatible with BLI. It's usually a good idea to start with a buffer system in which your proteins are well behaved.
- **Addition of 0.05% Tween 20 (or other surfactant) is usually required** to prevent non-specific binding, which is a frequent problem in BLI experiments.
 - Try detergent concentrations above the CMC, typically in the range of 0.02-0.1%.
- 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.
- 0.1% BSA can also be used to minimize non-specific binding.
 - ForteBio sells a detergent-based Kinetic Buffer (PBS + 0.02 % Tween20, 0.1 % BSA, 0.05 % sodium azide) that you might consider.
 - NOTE: BSA is not universally beneficial and can sometimes increase non-specific binding.

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 concentration ranges:
 - Load Sample (immobilized) 10-50 $\mu\text{g/ml}$ ($\sim\mu\text{M}$ range)
 - Analyte 0.01 – 100 $\times K_D$ (0.1 – 10 $\times K_D$)
- Sample and Reagent plate well volume
 - 96-well 200 μl
 - 384-well 80 – 120 μl
 - 384-well tilted bottom 60 μl

Getting Started

An Octet experiment involves multiple steps in which ForteBio Dip and Read Biosensor are moved between wells in microplates containing buffers, reagents and samples. The instrument can hold up to 96 sensors (one tray) for use in experiments with multiple assays, each using up to 16 sensors. Reagents and samples are placed in 96- or 384-well black microplates. Plate 1 is used in all experiments and can hold any/all sample or reagent types. Plate 2 is an optional reagent plate that cannot hold analytes use in the association phase.



Resources

Additional resources are available at the instrument, including Data Acquisition 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 should be less than 3 hr (10 % volume loss ~ 3.5 hours at 30 °C).

Data Collection

Startup

1. Book time on the PPMS calendar before you start.
2. Login to the computer using your PPMS credentials (eCommons ID and password).
3. The instrument should generally be left powered on at all times.
 - a. If it is not powered on, the power supply is on the shelf above the instrument.
4. Design your experiment in the Data Acquisition software before setting up your sample and reagent plates.
5. Open the instrument door with the Present Stage button in the software (green eject button). Do **NOT** pull on the thing that looks like a handle.

Experimental Data Collection

1. Start the Octet Data Acquisition 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. 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. To modify plate format, select Modify Plate:
 - i) Choose either 96-well or 384-well format for Plate 1 and Plate 2
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).
 - c. 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 (a reference sample well) to correct for a drift in the baseline
 - (1) Several sensor types have significant drift.
 - (2) A reference sample well is required for NTA.
 - d. 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) To create a new Assays, click New Assay or select all steps in Assay 1 and click replicate.
Modify the 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.
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, 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 a 96-well plate underneath with soaking buffer (usually running buffer) in the wells with sensors.
5. Review Experiment:
 - a. Click the arrow to review each assay step in your the experiment.
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. 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 min).
 - iii) Set temperature (30 °C minimum).
 - d. Advanced Settings:
 - i) Set Sensor Offset: 4 mm for standard well volumes (see table below for other offsets).
 - ii) Set Acquisition Rate: Standard Kinetics (5Hz, avg by 20).
 - 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.

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 (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

Data Analysis

Overview

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

Data Analysis HT new features:

- Data from multiple plates and/or experiments can be combined into one analysis.
- More flexible reference subtraction options are available for kinetic analysis.
- One-click raw data exports data into a csv table.
- Customizable report format.

Data Analysis Protocols

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.
 - (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.
 - iv) This step corrects for system artifacts (optical artifacts from buffer mismatch, etc.).
 - v) Choose a step to align (try dissociation first).
 - vi) The baseline before association and dissociation steps must be performed from the same well of a sample plate.
 - vii) Should not be performed with very fast on-rates as kinetic data may be lost.
- 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.
 - (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. 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 ForteBio 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!**

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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