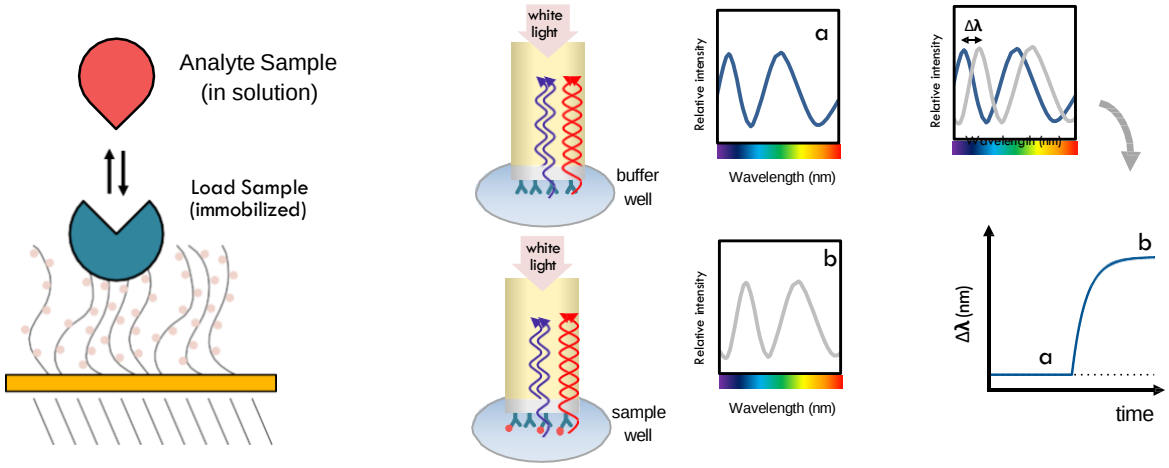
BLItz: Getting Started Guide to Biolayer Interferometry

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

MSI has a BLItz instrument from ForteBio, which measures binding kinetics and equilibrium by BLI using ForteBio dip and read biosensors in low volume.

Applications

- Kinetic binding: k_a, k_d
- Equilibrium binding: K_D
- Macromolecular interactions > 10 KDa
- Macromolecule quantitation

Key Features

- Disposable biosensors (sensor regeneration not required)
- Low sample volume: 4 µl sample

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.
- 96-well microplate to rehydrate sensors.
- 0.5 ml tubes (provided by MSI).
- An empty biosensor tray to use as a working tray for hydration (optional).
- Pipettes (provided by MSI).

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

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.
- The same tube of buffer should be used for the baseline and dissociation phase.

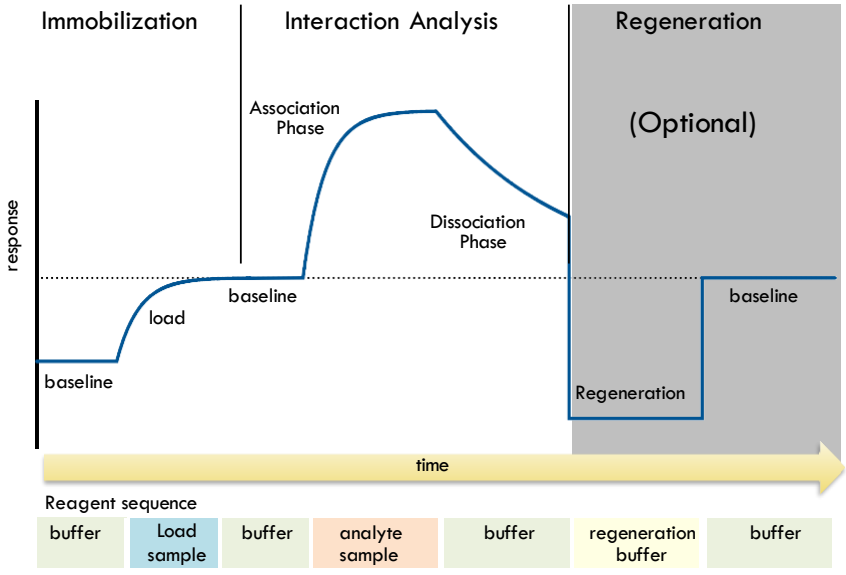
Samples

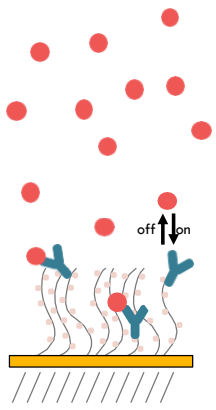
- All BLI experiments are setup with one molecule fixed to the biosensor surface (the Load Sample) and a second molecule in solution (the Analyte Sample).
- Concentration should be accurately measured
    - Errors in Load concentration can affect signal intensity

- 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 µg/ml (~µM range)
  - Analyte 0.01 – 100 X K_D (0.1 – 10 X K_D)
- Sample and reagent volumes
  - Sample is generally placed in the dropper but can also be place in a black tube
  - Buffer for baseline is placed in a black tube
  - Buffer for hydrating the sensor is placed in a 96-well plate (under the sensor tray)
    - Dropper volume 4 µl
    - Tube volume 250 µl
    - Plate volume 200 µl (Hydration)

Getting Started

A BLItz experiment involves multiple steps in which ForteBio Dip and Read Biosensor are placed in either a 4 µL drop holder or in a 0.5 mL tube (filled with 250 µL). Baselines, dissociation, and quench steps should be performed in a tube. Loading and association can be performed either in the drop holder (for steps ≤ 5 min, generally) or in a tube (for steps > 5 min). The droplet holder should be wash before and after use three times with 1M HCl and three times with your assay buffer.





Experimental Design Tips

- Do not overload the immobilized molecule.
- The same tube containing buffer should be used for the baseline and dissociation phase.
- The BLItz analysis will only perform a single reference sample subtraction (zero concentration of analyte). Use it.
- Do a control experiment with a reference sensor to measure non-specific binding to the sensor (using the highest concentration of analyte sample). If you see non-specific binding, then:
    - Optimize buffer conditions to eliminate non-specific binding.
    - Do a reference sensor measurement for each concentration of analyte and process and fit the data manually in a 3rd party fitting software package, such as GraphPad Prism.

General Care and Maintenance

Startup

1. Book time on the ACLS calendar before you start.
2. Login to the computer using your ACLS credentials (zID and password).
3. Turn on the instrument using the switch at the back.
4. Open the BLItz Pro software.
5. Choose an experiment type: almost all experiments will be Advanced Kinetics in which one molecule is immobilized and binding of another is measured.
6. For macromolecule quantitation, either association or load phase is used to measure the concentration. For same molecule, faster association rate means higher concentration.

Data Collection and Analysis

Advanced Kinetics Experiment

1. Preparation
    a. Hydrate the biosensors for at least 10 min before each measurement. Use the hydration tray and load 200 µL assay buffer in the bottom 96 well plate.
    b. Prepare protein samples in assay buffer (load 4 µL per measurement).
    c. Prepare assay buffer (250 µL in a 0.5 ml tube).
2. Input the Run Settings
    a. Include the molar concentration of the analyte sample for each measurement.
    b. Always enable the shaker.
    c. Set step types and duration. (For a first experiment, the default values are a good start.)
3. Click Next when ready to start the experiment.
4. Follow the prompts to switch between the buffer in the tube and samples in the drop holder.
5. Depends on the type of sensor, it can be generated with different buffers.
    a. Protein A and protein G sensors can be generated by 10 mM glycine pH 2.5 to pH 1.5
    b. Ni-NTA sensor can be generated by 500 mM imidazole in assay buffer

Data Analysis

1. Select the zero concentration of analyte as the Reference sample.
2. Enable step corrections at the start of association and dissociation.
3. Choose local or global fitting.
4. Click Analyze.

Shutdown

5. Clean drop holder.
6. Remove and discard biosensors and black tubes.
7. Return borrowed empty sensor trays to the drawer under the instrument.
8. Clean up in and around the instrument.
9. Turn off the instrument power.
10. Close the control and analysis software.
11. Logoff from ACLS