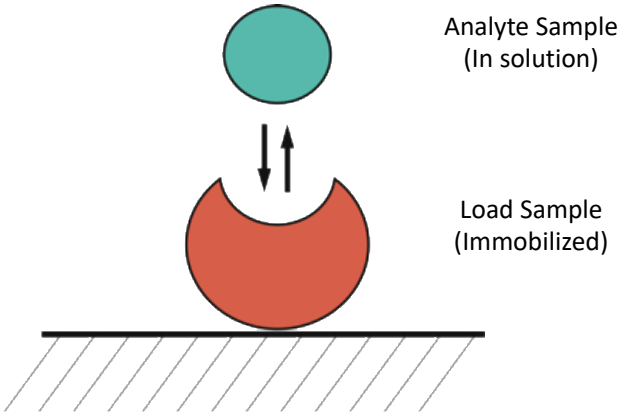
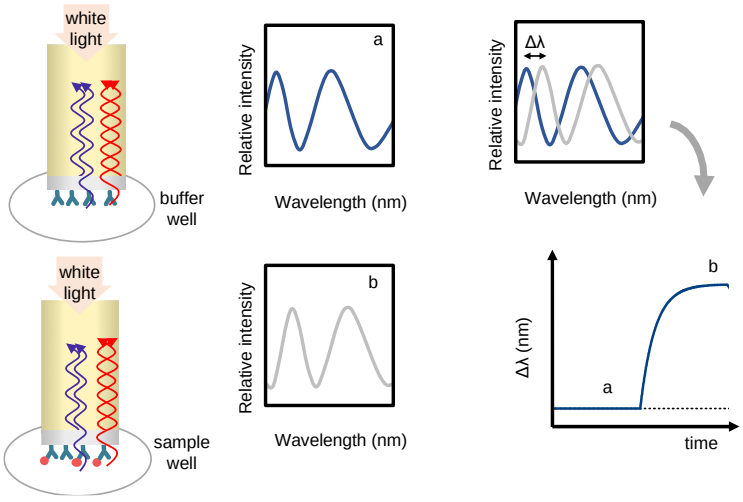
BLItz Getting Started

Introduction

The BLItz 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

Sample volume per measurement:	4 µl (dropper) or 250 µl (tube)
Ligand (to be immobilized) concentration	10-50 µg/ml (~µM range)
Ligand MW	≥ 10 KDa (to see it coupled to sensor)
Analyte concentration	0.01-100xK_D (maximum ~ 10 µM)
Analyte MW	≥ 10 KDa

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**. Ideally, the sample used for the association phase is in a buffer identically matched to the buffer used for the baseline and dissociation. Immobilized ligand should also be in the same buffer, if possible. The same tube of buffer should be used for the baseline and dissociation phase, if a baseline correction is performed.

Addition of 0.02% (0.005%-0.1%) Tween 20 or other detergent, will almost always be 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).

Designing first experiment

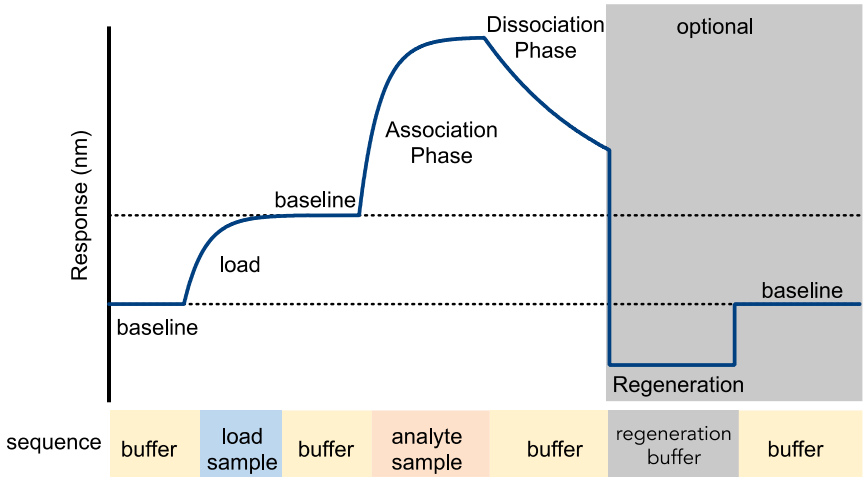
A BLItz experiment is divided into steps in which the Dip and Read Biosensor is placed in either a 4 µl drop holder in a 0.5 ml tube (filled with 250 µl). Baselines, dissociation, and quench steps should be performed in a tube. Loading and association can be performed either in the drop holder (for steps ≤ 5 min, generally) or in a tube (for steps > 5 min).

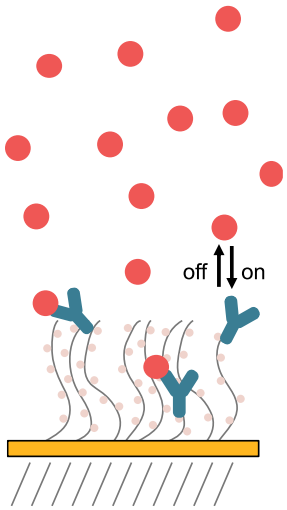
Supplies

BLItz general supplies	**Supplier**	**Part Number**
Greiner 96-well flat-bottom plate	VWR	82050-784
Black 0.5 ml tubes	VWR	80078-648

Dip and Read Biosensors* (96/tray)	**Supplier**	**Part Number**
Streptavidin (SA) biosensors	Forte Bio	18-5019
anti-His (HIS1K) biosensors		18-5120
Ni-NTA (NTA) biosensors		18-5101
anti-GST biosensors		18-5096
Super-Streptavidin (SSA) biosensors		18-5057
Protein A biosensors		18-5010

*see ForteBio website for more sensor types: http://www.fortebio.com/biosensor-types.html





Experimental Design Tips

- Don't overload the immobilized molecule.
- 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, you'll need to do one or more of the following:
    - Optimize buffer conditions such that there is no 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.
    - Switch to the Octet RED384 instrument and do reference sensor subtraction.

Starting the Instrument

1. Turn on the instrument using the switch at the back.
2. Open the BLItz Pro software.
3. Choose an experiment type. Almost all experiments will be Advanced Kinetics (in which one molecule is immobilized and binding of another is measured).
4. Consult the BLItz User Guide for detailed experimental instructions.

Setting up an Advanced Kinetics Experiment

1. Preparation
    a. Hydrate the biosensors for at least 10 minutes before each measurement
    b. Prepare protein samples in assay buffer (load 4 µl per measurement)
    c. Prepare assay buffer (250 µl in a black tube)

2. Input the Run Settings
    a. Include the molar concentration of the analyte sample for a each measurement.
    b. Always enable the shaker.
    c. Set step types and duration (the default is reasonable first experiment)
3. Click Next when ready to start the experiment
4. Follow the prompts to switch between buffer in the tube and your samples in the drop holder.
5. Data Analysis
    a. Select the zero concentration of analyte as the Reference sample
    b. Enable step corrections at the start of association and dissociation
    c. Choose local or global fitting
    d. Click Analyze.

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