Introduction

The 16-channel Octet RH16 system uses biolayer interferometry (BLI) to characterize biomolecular interactions in real time, giving insight into desirable kinetic parameters such as k_{on}, k_{off} and k_d for given interactions. Additionally, the Octet can be used for applications ranging from protein quantitation, lead identification/optimization, antibody characterization and cell line development.

Features

Dip and Read Technology

The biosensor tip is coated with a compatible matrix that minimizes non-specific binding while providing a uniform and non-denaturing surface for biomolecules.

Wide range of samples

The Octet is suitable for use with purified samples as well as unpurified samples (including whole cells) with multiple complex matrices.

Label free study of proteins

Samples do not need to be chemically labelled for detection, allowing for the study of samples in their native context, giving data that is more representative of conditions *in situ*.

Low sample volume options

50 µL per well in 384-well tilted bottom microplate
200 µL per well in 96-well microplate

Fast, simplified workflows

Workflows can be simplified with automated steps for high-throughput analysis with up to16 channels in 384-well microplates or 8 channels in 96-well microplates.

Non-destructive investigation of samples

Given the non-invasive nature of BLI, samples can be recovered and used for further experiments, thereby cutting down on the amount of protein needed.

User friendly software

Software allows for modification of protocols during runs and makes data processing straightforward.

Structural & Biophysical Core Facility

Greg Wasney

Manager, Structural & Biophysical Core Facility
Peter Gilgan Centre for Research & Learning
The Hospital for Sick Children
686 Bay Street, Rm. 21.9708
Toronto, ON. M5G 0A4

Email: *greg.wasney@sickkids.ca*

Office: 416.813.7209
Office Internal Ext. 307209
Lab: 416-813-7654 ext. 309442

http://lab.research.sickkids.ca/sbc-facility/

SickKids
THE HOSPITAL FOR SICK CHILDREN

Produced by Serban Popa and Shina Hussain

The Hospital for Sick Children's

Structural and Biophysical Core Facility

Octet RH16 Biolayer Interferometry- Sartorius (ForteBio)

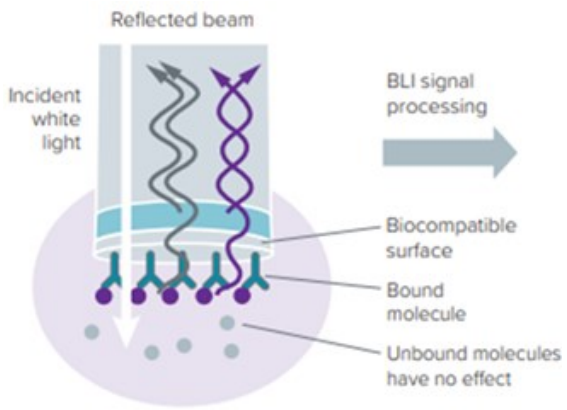
Biomolecule Quantitation and Molecular Interaction Kinetics

- Determine k_{on}, k_{off}, k_d for desired bimolecular interactions in real time.
- Detection and quantitation of analytes ranging in size from hundreds of nm (bacteria/viruses) to low nm (antibodies/peptides/compounds).
- High throughput analysis of samples with up to 16 parallel channels in low-volume 384-well plates or up to 8 parallel channels in 96-well plates.
- Wide variety of biosensor tips permitting multiple ways to immobilize target sample.

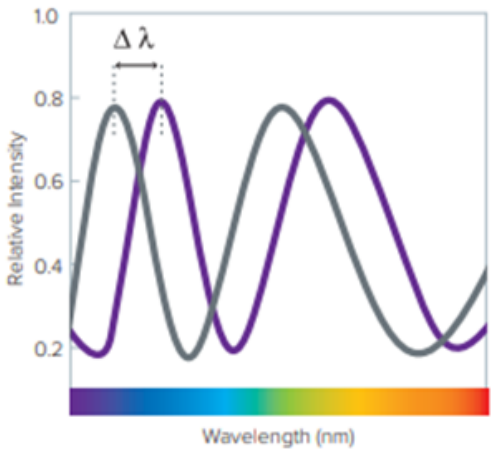
Octet RH16 Biolayer Interferometry

Theoretical Background

Biolayer Interferometry is an optical technique that detects changes in interference patterns from light waves. A biomolecule of interest is immobilized onto the tip of a biosensor and light interference at the tip of the biosensor is measured. The incident light reflects through two layers: a biocompatible layer and an internal reference layer.



Biological interactions will alter the thickness of the biolayer at the biosensor tip, thus altering the way the light reflects, resulting in a spectral shift that can be detected and monitored via a sensogram. The resulting spectral shift is then measured and can provide real time insight into the kinetics of the molecular interaction.



Example workflow

Below is a summary of a typical Octet experiment.

1) Loading is the immobilization of one binding partner (ligand) using the appropriate immobilization strategy.

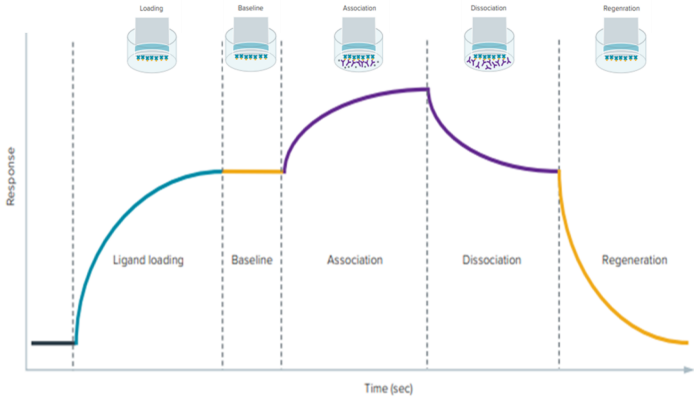
2) Baseline is the washing away of any loosely bound ligand to equilibrate the biosensor tip.

3) Analyte association introduces the ligand's binding partner in order to obtain association kinetics.

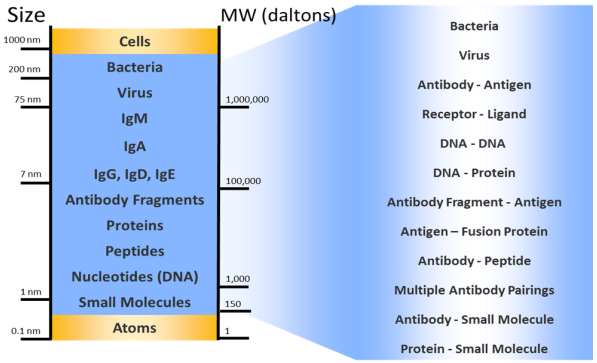
4) Dissociation of the analyte from the formed complex obtains dissociation kinetics.

5) Regeneration of the biosensor removes bound ligand to allow for further experiments if applicable (such as when using Ni-NTA tips).

Idealized data for what one should expect to see for a typical kinetics experiment is shown below. Plotted is the response (the magnitude of the wavelength shift as a result of binding to the biosensor) as a function of time. The resulting data can then be fit to appropriate models to get the desired information.



Applications and size ranges compatible with Octet



Immobilization strategies

The table outlines a selection of strategies (others are available) for immobilizing samples based on sample features.

Sensor	Description	Mechanism of action
AR2G	Amine Reactive 2G	Binding to lysine residue of sample
NTA	Ni-NTA	Nickel binding to His tags
SA	Streptavidin	Binding to biotin tagged sample
SSA	Super Streptavidin	Binding of biotin tagged protein for small molecule screening

Technical properties of the Octet RH16	
Sample volume	50 - 200 µL
Microplate types and Position	2 x (96-or 384-well)
Analysis temperature	15 - 40 ℃
Association rate constant (k_a)	10^1 to 10^7 $M^{-1}S^{-1}$
Dissociation rate constant (k_d)	10^{-6} to 10^{-1} S^{-1}
Affinity constant (K_D)	1 mM – 10 pM
Molecular weight detection	>150 Da