OctetRED96e
Getting Started Guide

SPR/BLI Platform

Institut de Biologie Structurale (IBS)
Integrated Structural Biology, Grenoble

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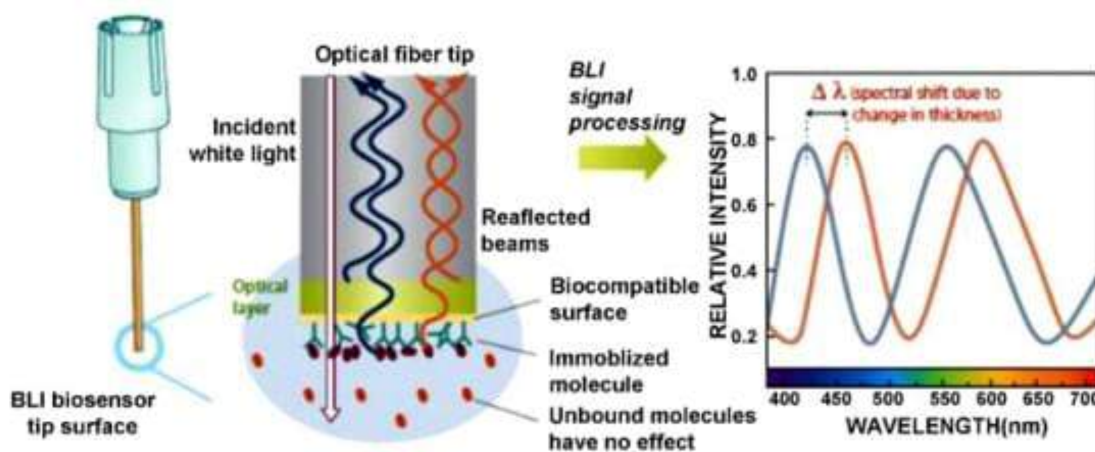
INTRODUCTION

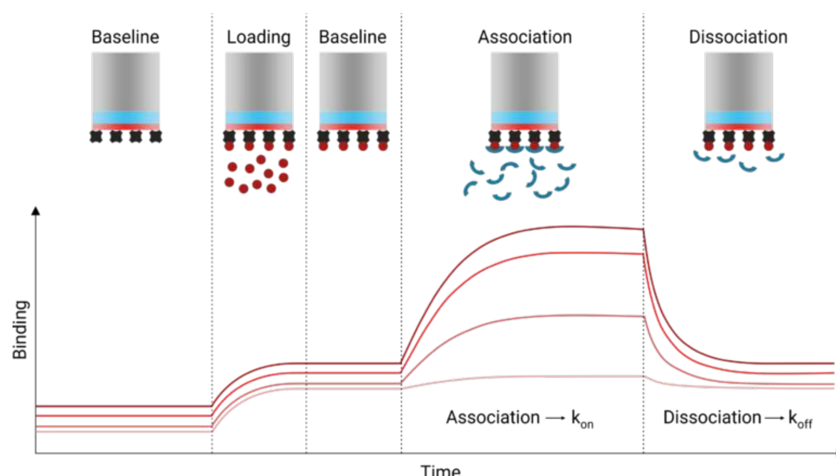
The OctetRED96e is an instrument for Biolayer Interferometry (BLI), an optical technique that measure the thickness of a biolayer by the analysis of the interference pattern of white light reflected from two surfaces. The BLI technique allows real-time detection and monitoring of biomolecular binding events.

In a typical BLI experiment, one molecule (**Ligand**) is immobilized or captured to a Biosensor and binding of a second molecule (**Analyte**) is measured when biosensors are dipped in the shaken sample solution. BLI can be then used to measure equilibrium binding constant (affinity, K_D), Kinetic binding rate constants (k_a, k_d).

BLI is also used for analyte quantification and ELISA types.



Response is measured in real-time as spectral shift (nanometers - nm) and displayed on a Sensorgram. It is directly related to ratio between the biolayer thickness and its refractive index, then to the thickness of biomolecule layer bound to the surface.



Major steps in a BLI experiment have to be carefully prepared and performed, and each one will have to be optimized for your experiments:

- Sample preparations are crucial. Biomolecular samples must be:
  - Homogeneous,
  - Highly pure,
  - Stable over time and at the analysis temperature (default 25°C).
- Capture or Surface preparation:
  - The Ligand will be directly immobilized or captured via a secondary molecule to the Biosensor (see further for strategies).
- Interaction analysis:
  - During association phase, the Analyte is put in contact with the functionalized surfaces. Contact time and shaking speed have to be adjusted (see further for strategies),
  - During dissociation phase, the Analyte is washed out by dipping biosensor in dissociation buffer (see further for strategies),
  - Several experiments at different concentrations of Analyte is necessary to determine binding constants (see further for methods).
- Regeneration:
  - In order to be reused, the functionalized surfaces must be regenerated at the end of each cycle by removing remaining bound Analyte or by removing Ligand (see further for strategies).

FACILITY ACCESS, LOCALIZATION AND CONTACTS

Access

SPR/BLI platform is opened to:

- Academics according to term to be defined (service/autonomous access or collaboration) depending of the involvement of the platform personal.
- Industrials as full service (contact platform for details and quotes).

Localization

- The SPR/BLI platform is located on EPN campus-Grenoble, in the IBS building, room 515: http://www.isbg.fr/contact/article/access
- External users have to demand authorization to access the EPN campus. Contact platform personal prior your visit.
- External users can request accommodations at the EPN campus Guest house (at their own costs). Contact platform personal prior your visit.

Use of the facility

New users

Each novel user must contact platform personal prior any experiments and instrument bookings.

A preliminary meeting is mandatory to:

- Define terms of platform usage: collaboration or autonomous access.
- Discuss of the experimental conditions and define the best strategy to carry out the study.
- Schedule training & the first use of the instrument.

Each novel user has to follow instrument and platform usage training and must agree to comply with the general conditions of use and to follow operating instructions.

Trained users

Trained and registered users have to check booking calendars on SPR/BLI platform webpage (http://www.isbg.fr/biophysics-characterisation/bli/) and can reserve the instrument via the reservation form.

Samples and buffers sending & returning

External users can send their samples and buffers to the platform via the carrier of their choice (DHL, Fedex...) at their own cost.

Refer to Contacts section for full postal address and contact details.

Inform platform personal with carrier name, tracking number and parcel contents.

Platform personal can return samples and buffers in the same way at your own cost.

Consumables and kits ordering

Users must provide consumables specific for their experiments (see What to bring and What the platform provides sections).
Consumables must be ordered to Molecular Devices/Fortébio (https://www.moleculardevices.com/products/biologics).
No other Biosensors will be accepted for the instrument.

Contacts

Address
SPR/BLI Platform
Institut de Biologie Structurale - UMR 5075 (CNRS-CEA-UJF)
71 Avenue des Martyrs
CS 10090
38044 Grenoble cedex 9
FRANCE

Platform webpage
http://www.isbg.fr/biophysics-characterisation/bli/

Scientific responsible
Jean-Baptiste REISER
Email address: jean-baptiste.reiser@ibs.fr
Phone #: +33 (0)4 57 42 85 49

Technical/Platform administration assistance
Anne Chouquet
Email address: anne.chouquet@ibs.fr
Phone #: +33 (0)4 57 42 85 47

ISBG administration
Darren Hart & Linda Ponnet
Email address: linda.ponnet@isbg.fr
Phone #: +33 (0)4 76 20 94 48

SAMPLE AND BUFFER PREPARATION

Sample and buffer qualities have to be carefully checked to insure to reduce any artefacts, unspecific bindings, undesired signals...

Ligand

- Ligand samples may be prepared homogeneous and stable over experimental time and at the analysis temperature. High biochemical purity (>95%) is recommended for direct immobilization methods (*i.e.* amine coupling). Partially purified samples or clarified crude samples can be used with secondary capture.
- Ligand samples must be prepared in buffer compatible with immobilization chemistry (*i.e.* avoid Tris buffer and prefer low ionic strength for amine coupling).
- Required Ligand concentration and amount will vary based on the immobilization and capture strategy. Typically, 200 µL at 2-50 µg/ml per immobilization are necessary. Highest concentration may be necessary depending of the coupling method.
- Ligand samples must be filtered (0.22 µm) or cleared by centrifugation (16 kg, 5-10 min) before experiments.

Analyte

- Analyte samples must be homogeneous, soluble and stable at the analysis temperature and over experiment time.
- Analyte samples are recommended to be prepared in analysis buffer (same batch or minimum requirement: same composition). Perform dialyses or exchange buffer chromatography in the running buffer batch that will be used for your experiment.
- Accurate concentration measurements of Analyte samples are mandatory since kinetics rates and/or affinity determination rely on the free concentration. Use a nanodrop type UV/visible-spectrophotometer.
- Required Analyte concentrations will depend on the affinity (K_D) of the interaction.
- Required volume per well is 200 µL.
- Analyte samples must be filtered (0.22 µm) or cleared by centrifugation (16 kg, 5-10 min) before experiments.

Running buffer

- Choose an analysis buffer compatible and in which your samples behave well (PBS, HBS, TBS...).
- Addition of 0.002% to 0.1%Tween-20 is required to prevent non-specific binding.
- Addition of 0.01%w/v BSA may be needed to prevent non-specific binding.
- 100 mL to 250 mL of analysis buffer are required.
- Commercial commonly used buffers can be purchased from Molecular devices/Fortébio.
- Buffer are recommended to be filtered (0.22 µm).

EXPERIMENTAL DESIGN

Immobilization/capture

The first step in the design is the Ligand immobilization or capture. A proper surface preparation is one of the keys for the success in measuring kinetics and/or affinity since lot of bias and artefacts can originate from the immobilization.

What strategy?

BLI offer several methods and chemistries to immobilize or capture Ligands over a surface. The strategy would be defined depending of the biochemistry of the Ligand and the interaction (Molecular weight, pI, purification tag, interaction type...):

- Direct and irreversible immobilization: Amine coupling (covalent).
- Secondary and irreversible capture: Streptavidin/biotin (fM affinity).
- Secondary and reversible capture: Ni-NTA/His-tag (nM affinity), Antibody capture (nM affinity).

What sensor Chip?

Molecular devices/Fortébio sells several types of Biosensors with different immobilization/capture chemistries.
The commonly used are:

- AR2G (Amine Reactive Second Generation) for amine coupling (irreversible)
  - Efficient amine coupling depends on the Ligand concentration (5 to 100ug/ml) and must be performed at a lower pH than the Ligand pI (> 1 pH unit below),
  - Perform a pH Scouting to determine suitable concentration and pH for amine coupling immobilization.
- NTA (Ni-NTA) for His-tagged Ligand captures (reversible):
  - 6His-tagged Ligand can be unstable,
  - Easily regenerable with EDTA or EGTA (no feasible if extra coupling).
- SA or SAX (Streptavidin) for capture of biotinylated Ligands (irreversible):
  - Ligands must be biotinylated (several strategies: Biotin tags, cross-linking...).
- Antibody capture (reversible):
  - Several biosensor types are available (His, Antibody capture),
  - Easily regenerable with Glycine.

What immobilization level?

- For each interaction, a rough estimation of ligand level can be calculated (R_{ligand}) according to:
  - The maximum Analyte response you expect (R_{max}),
  - The Ligand/Analyte Stoichiometry (V),
  - The Ligand/Analyte molecular weight (MW) ratio,
  - Percentage of remaining active Ligand ($\%_{active\ ligand}$),

$$R_{ligand} = R_{max} \cdot \frac{MW_{ligand}}{MW_{analyte}} \cdot \frac{1}{V} \cdot \%_{active\ ligand}$$

- For protein/protein interaction, set R_{ligand} such that R_{max} is 1 to 5 nm.

- For direct immobilization (*i.e.* amine coupling), remaining active Ligand may between 25 to 75% of R_{ligand}. For secondary capture, 100% of active molecules is assumed.
- Be careful, local concentration at the surface can be high.
- Low density is always better! It minimizes steric hindrance, aggregation at the surface and mass transport limitation.

Interaction analysis

Interaction analysis needs to be carefully designed to be able to analyze data and retrieve reliable interaction parameters.

Pilot analyses, what for?

Prior any full kinetic experiment, pilot analyses may be performed to test and optimize conditions and parameters such as:

- Shake speed.
- Association time (contact time).
- Dissociation time.
- Analyte sample concentrations.
- Analysis buffer composition (unspecific binding).
- Regeneration.

What Shake speed?

- Recommended shake speed for kinetic assays is minimum 1000 rpm for quantification 400 rpm.
- shake speed is important for minimizing mass transport limitation. In case of mass transport, increase the speed (up to 1500 rpm).
- Faster is better! (1000 rpm is the standard).

What association/contact time?

- The contact time needed depends on the kinetics rates and the Analyte concentration. It should be long enough to have sufficient curvature and/or reach equilibrium.
- Typical protein/protein interaction needs 1 to 10 min of contact time.

What dissociation time?

- The measured dissociation has to be long enough to obtain a reliable estimation.
- At least 5% of signal decrease is needed.
- $t_{5\%} = 0.052/k_d$

What Analyte concentration?

- Analyte sample concentration must be within a range of 0.1 to 10 times the K_D.
- Interaction affinities can span from sub-nanomolars to hundreds of millimolars. You may come with an idea.
- A good start is in the micromolar range, then scan concentrations.

What reference and unspecific binding?

- Unspecific binding (Reference sensors):
    - A pilot analysis with a "empty" biosensor (with no ligand captured) is recommended to be performed with an analyte concentration between the K_D and ten times the K_D in order to evaluate the unspecific binding of analytes to the biosensor surface,
    - In case of unspecific binding, addition of higher Tween-20 (up to 0.02%) and/or BSA (up to 0.1%w/v) concentrations can be tested in order to lower the unspecific binding. Changing salt concentration and/or pH of the analysis buffer can also help,
    - In case of analyte unspecific bindings (less than 25% of the total binding signals), parallel reference biosensors need to be prepared and the unspecific signals subtracted to binding signals,
    - Several types of reference surfaces can be done:
        - Minimal surface is « Activated/Saturated » surface (treating with the same procedure but omitting the Ligand),
        - Include the capturing molecules,
        - Dummy or irrelevant Ligand can be use (scrambled peptide, BSA, inactive mutant) to mimic the active surface as closely as possible.
- Bulk contribution and drift of instrument baseline (Reference sample/well):
    - Always use an analyte zero-concentration (a functionalized sensor but no analyte during association). The bulk contribution, noise and instrument baseline drift (0.05 to 0.1nm/hour) will be then subtracted to binding signals.

What regeneration conditions?

Proper regeneration conditions have to be found to remove Analyte molecules from the functionalized surface. They consist of dipping the biosensors in denaturing agents and need to be determined empirically.

- 2 different strategies are employed:
    - Complete removal of the Analyte without damaging the Ligand can be applied for all immobilization methods,
    - Complete removal of the Ligand between cycles can be applied only for reversible immobilization methods (highly reproducible capture of the Ligand is mandatory).
- Regeneration may not be needed if k_d is fast (5-20 min off-time).
- Optimization:
    - A regeneration kit can be provided by the platform for your first assays,
    - Use different regeneration buffers in series and during pilot analysis,
    - Combination of different regeneration can be applied,
    - Test in order:
        - Any buffer you know that can disrupt the interaction (i.e. EDTA, competitors...),
        - High salt (NaCl 1M, 2M, 3M; $MgCl_2$ 1M, 2M, 3M),
        - Low pH (HCl 1mM, 5mM, 10mM; 10mM Glycine pH 3, 2.2, 2, 1.5),
        - High pH (NaOH 1mM, 5mM, 10mM),
        - SDS (0.01%, 0.02%, 0.05%, 0.1%, 0.5%).

- More pilot analyses are mandatory after regeneration to assess the activity of the Ligand (manual or surface performance wizard).

What minimum requirement for kinetics assay?

- At least 5 different concentrations of Analyte – best: 7 or more concentrations.
- Spanning a concentration range of 0.1-10 * K_D
- Samples must be diluted in analysis buffer and in dilution series (1.5 to 3).
- 1 zero-concentrations (buffer only) for reference may be included.

Evaporation?

- Samples and reagents evaporations occur during experiment: 10% volume lost in 3 to 4 hours depending of the plate temperature.
- Try to design experiment lasting less than 2 to 3 hours.
- If experiments are longer to 3 hours, use an evaporation cover to reduce the phenonum.

Replicates?

Replicates may be needed to assess the reproducibility of the assays and to create a sound statistical basis of the obtained values:

- Include at least one replicate or more in the kinetics series to evaluate stability and reproducibility of the assay.
- Because the error given by the fitting program will give only the statistical error of the fits, true replicates of the assay may be performed:
  - At minimum, re-do experiments with same samples and same functionalized surfaces (“within-experiment” replicates),
  - Best is to re-do assays with all new fresh samples (“experimental” replicates: new chip, new samples lots...).

WHAT TO BRING FOR YOUR EXPERIMENTS

The minimum requirement is:

- Analysis buffer(s) in glass bottles: 150 to 500 mL.
- Ligand samples at the highest concentrations (to be diluted in immobilization buffers).
- Analyte samples at the highest concentrations (to be diluted in analysis buffers).
- Regeneration buffer if known.
- Biosensors: no other biosensorchips than from Molecular Devices/ForteBio will be accepted. Some biosensors are available at the platform.
- Pipettes and tips (best if controlled and calibrated).
- Necessary plastic wares (Falcon, Eppendorf type tubes).
- Any previous and relevant data and/or protocols.

WHAT THE PLATFORM PROVIDES

The SPR/BLI platform makes available the basic and common supplies for experiments on the OctetRED96e:

- OctetRED96e instrument.
- 4°C refrigerator and -20°C freezer for sample storage.
- Bench vortex and centrifuge.
- P20/Tween-20 at 10% stock (added to your analysis buffers prior experiments – located on the top shelf).
- BSA at 10%w/v stock (added to your analysis buffers prior experiments – located in the -20°C freezer).
- pH Scouting and Immobilization kits for amine coupling methods (located in the 4°C refrigerator and in the -20°C freezer).
- Regeneration scouting kit (located in the 4°C refrigerator).
- Black polypropylene 96-wells plates (on the bench and in the drawers).
- Evaporation cover (in the drawers).
- External users who can't bring bench supplies can borrow supplies from our lab (pipettes, tips, plastic and glass ware). Contact platform personal.
- If needed, a nanodrop UV/visible-spectrophotometer is available from our lab (room 520).

HOW MUCH TIME DO I NEED

The time needed to fully characterize your interaction would be very dependent of your biological system, the strategy employed, the time required to optimize your experiments and any difficulties that you can encounter... It can range from days to weeks...
Here are some estimations of time required for classical methods:

- Start-up sequence: 5 min.
- pH Scouting prior amine coupling immobilization: 30 to 60 min per ligand sample.
- Amine coupling immobilization: 45 to 60 min per ligand sample.
- Secondary capturing: 20 to 30 min per ligand sample.

- Pilot analyses: ½ day to several days depending on number of samples at different concentrations, test of several contact and dissociations times, of regeneration conditions.
- Kinetics analysis: 1/2 to several hours depending on method and concentrations.
- Shutdown sequence:5 min.

START-UP SEQUENCE

- Before you start, check booking calendar on BLI platform webpage (http://www.isbg.fr/biophysics-characterisation/bli/) and reserve the instrument by filling the reservation form on the web page.
- Login to the computer using your IBS/LDAP credentials.
- Create a subdirectory in which result data files will be saved:
    - Open a Windows navigation window,
    - Navigate into ***Desktop → Bia Users – Shortcut***,
    - Select ***New folder***,
    - Change newly created folder name by your name.

  Or:
    - Navigate into ***Documents***,
    - Select ***New folder***,
    - Change newly created folder name by your name.
- Check that no sensor tray in place (left tray).
- Check that no sample plate is in place (right tray).
- Insure that the instrument door is closed.
- Start the ***Data Acquisition software*** (link on the Desktop or in Windows menu).
- Wait the instrument to be ready.

RUNNING EXPERIMENTS

Building Method from Wizard Templates

Several basic experiments can be run from templates implemented in the Data acquisition software.

To start a new template:

- Select ***Experiment → New Experiment Wizard Template…***

  Or

  Click on the ***Magic Wizard Icon.***
- Select an ***Option to start*** (for details, consult OctetRED96e manuals):
  - New Quantification Experiment:
    - Basic Quantification: for measuring analyte concentration with ELISA-like assays,
    - Basic Quantification with regeneration: for measuring analyte concentration with ELISA-like assays but reusing biosensors,
    - Advanced Quantification: for more complex methods,
  - New Kinetics Experiment:
    - Basic Kinetics: for simple scouting, analysis of association and dissociation and screening using several types of Immobilization/capture chemistries,
    - Epitope Binning: for competitive immunoassay to characterize analyte library against a target ligand.
- Select one of the ***Available Templates***.
- Click on the ***Green Arrow*** to open the method.
- Modify the method if needed (see *Building Method from Blank Experiment* for more details).
- Open the instrument door.
- Prepare your samples and reagents in a black 96-wells plate according to your reagent plate map:
  - Add 200µL of each reagent in each well,
  - Place the plate on its holding position in the instrument (right position) and insure it is well hold.
- Prepare your biosensors:
  - Fill a black 96-wells plate with 200µL hydration buffer per well and place it at the bottom of a biosensor tray (dark blue part),
  - Transfer biosensors from their storage tray to the top of the tray (green part) dedicated to the instrument. Be careful not to shock or damage the biosensors,
  - Assemble the top of biosensor tray (green part) to the bottom of the biosensor tray (dark blue part),
  - Place the full tray on its position in the instrument (left position),
- For long experiments (over than 2 hours), an evaporation cover can be added over the sample and reagents plate. When placing it in the instrument, insure its legs are well inserted to their position (blue light must be on continuously).
- Close the instrument door.
- Start the method.

Building Method from blank

Method can be built and run from scratch in the Data acquisition software.
To start creating a new template:

- Select ***Experiment*** → ***New Experiment Wizard Template…***
  Or
  Click on the ***Magic Wizard Icon.***
- Select an ***Option to start*** (for details, consult OctetRED96e manuals):
  - New Quantification Experiment:
    - Basic Quantification: for measuring analyte concentration with ESILA—like assays,
    - Basic Quantification with regeneration: for measuring analyte concentration with ESILA—like assays but reusing biosensors,
    - Advanced Quantification: for more complex methods.
  - New Kinetics Experiment:
    - Basic Kinetics: for simple scoutings, analysis of association and dissociation and screenings using several types of Immobilization/capture chemistries,
    - Epitope Binning: for competitive immunoassay to characterize analyte library against a target ligand.
- Select ***Blank Experiment*** in the ***Available Templates***.
- Click on the ***Green Arrow*** to open the blank method.
- 5 steps represented by 5 tabs to be followed in order are necessary to create a method:
  - ***1-Plate Definition*** to create samples and reagents plate map:
    - On the plate schematic, click either on a single well, or on a group of wells (click & drag), or on a column number to select the desired wells,
    - Right click on the selected wells and choose one of the reagent categories:
      - *Sample*: for analyte samples,
      - *Reference:* for analyte or reagent samples used for double referencing,
      - *Control, Negative or Positive control*: for analyte samples used for controls,
      - *Buffer*: for analysis buffer (baseline, dissociation…),
      - *Activation:* for s-NHS/EDC mix used for amine coupling Immobilization,
      - *Quench*: for Ethanolamine used for amine coupling saturation,
      - *Load*: for ligand samples,
      - *Wash*: for wash buffers,
      - *Regeneration & Neutralization*: for regeneration and neutralization buffers,
    - Fill the right table with samples and reagents information (ID, concentration, Molecular weights, Additional information),
    - Change concentration units if necessary,
    - Click on the left or right green arrow to go to the next step,

- ***2-Assay Definition*** to create your analysis method***:***
  - First, create a list of the steps needed for your method in the first table (top right). Click ***add*** and select any or several of the followings and click ***OK*** to add them in the table:
    - *Association*: for the association/contact time between your functionalized biosensors and your analyte samples,
    - *Dissociation*: for the dissociation step (contact between biosensors and analysis buffer),
    - *Baseline*: for measuring the baseline before the association step (contact between biosensors and analysis buffer),
    - *Loading*: for the Immobilization/capture of your ligands (contact between activated biosensors and ligand samples),
    - *Activation*: for activation biosensors with activating reagents (typically AR2G biosensors, sNHS-EDC mix for amine coupling methods),
    - *Quenching*: for saturation of the biosensors after Activation and Loading (typically AR2G biosensors, Ethanolamine for amine coupling methods or SA/SSA/SAX biosensors, biocytin quenching),
    - *Regeneration*: for regeneration and neutralization of your biosensors,
    - *Custom*: for any extra steps,
    - *Dip*: for dipping biosensors,
  - Change Names, Times and Shake speeds if needed,
  - Second, create an or several assays (group of ordered steps with plates information). Add the steps one by one and in the final order to create your methods into the second table (bottom right):
    - Select the associated samples or reagents wells (click on the column number from the plate map),
    - Select the step (double-click on the line of it in the first top table). The new method step is then created in the second table,
    - Repeat with your next steps until the end,
    - To create new assays: click ***New Assay*** or select all steps in your previous assay and click ***Replicate***,
  - Change Sample numbers, Step Names, Sensor types and add comments if necessary,
  - You can create several assays, remove, move up and down steps by clicking on the top buttons,
  - Click on the left or right green arrow to go to the next step or to go back to the previous editing step,
- ***3-Sensor Assignment:***
  - Automatically, the software fills and auto-assigns the Sensor tray positions with the biosensors needed for your method and color codes them accordingly to your assays,
  - You can change Sensor type if needed,

- To move biosensors on the map, right click on the selected biosensors and select remove. The biosensors will be moved on the next available positions on the map.
- Select or unselect ***Replace Sensor in the tray after use***, depending if you want or do not want to save and reuse the biosensors,
- Click on the left or right green arrow to go to the next step or to go back to the previous editing step,

- ***4-Review Experiment:***
  - Both sample & reagent plate and biosensor tray maps, as well as the assay step table are displayed,
  - Click on either left or right black arrow to navigate in the different method steps. In black circles, the sample & reagents wells, biosensors positions and step are highlighted at each step,
  - Click on the left or right green arrow to go to the next step or to go back to the previous editing step,
- ***5-Run Experiment:***
  - Fill and change the different fields:
    - *Data repository*: Fill with your own saving data folder name,
    - *Experiment run name (sub directory)*: Fill with a name of a subdirectory where the data files will be stored,
    - *Plate Name/barecode*: fill with the prefix of data file names,
  - Check or uncheck the following options:
    - *Delay experiment start*: if you need a waiting time (600s by default) in case of biosensors hydration,
    - *Shake sample plate while waiting*,
    - *Open runtime charts automatically and Automatically save runtime chart*: to display and save sensorgrams in real time,
    - *Set plate temperature* (by default 25°C): the sample and reagent plate can be thermalized between 15 and 40°C,
    - *Hold plate temperature after run,*
  - Click on the left green arrow to go back to the previous editing step.

- Open the instrument door.
- Prepare your samples and reagents in a black 96-wells plate according to your reagent plate map:
  - Add 200µL of each reagent in each well,
  - Place the plate on its holding position in the instrument (right position) and insure it is well hold.
- Prepare your biosensors:
  - Fill a black 96-wells plate with 200µL hydration buffer per well and place it at the bottom of a biosensor tray (dark blue part),
  - Transfer biosensors from their storage tray to the top of the tray (green part) dedicated to the instrument. Be careful not to shock or damage the biosensors,
  - Assemble the top of biosensor tray (green part) to the bottom of the biosensor tray (dark blue part),
  - Place the full tray on its position in the instrument (left position),

- For long experiments (over 2hours), an evaporation cover can be added over the sample and reagents plate. When placing it in the instrument, insure its legs are well inserted to their position (blue light must be on continuously).
- Close the instrument door.
- Click on ***GO*** to start the method.

SHUTDOWN SEQUENCE

- Remove all sensors from the dummy sensor holder plate.
- Remove any sensor plate and sample plate from the instrument.
- Report any problems to the personal and in the logbook.
- Wipe the detector heads with a dry Kimtech tissue
- ***FILL THE LOGBOOK***
- ***CLEAN THE BENCH***
- Close the ***Data Acquisition Software***.
- Logoff from the computer.

DATA SAVING AND ARCHIVING

The SPR/BLI platform does not guarantee saving and archiving of user data.
SAVE and ARCHIVE your data and evaluation procedures before leaving the platform.

For internal IBS users

- Login to the computer using your IBS/LDAP credentials.
- Your IBS internal network directory may be mounted automatically at login.
- Open a Windows navigation window.
- Navigate into the directory where you have saved your data:
    - ***Desktop → Octet Users – Shortcut***,

    Or
    - ***Documents → your directory***
- Copy your subdirectory or data files,
- Navigate into ***Network→ science4 → <your LDAP name>***
- Paste your Biausers subdirectory or data files into your network folder.
- Logoff from the computer.

For external users

- Ask for instructions to the platform personal for a secured data transfer.

IN CASE OF TROUBLE

Suspected bad sensorgram

- Check the sensors and plates: broken sensors? Good positioning? Right sensors?
- Check the samples and plates: Good positioning? Right samples? Right volumes?
- Check the instrument: are the detector lights on?

Data acquisition program stops with error or hangs

- Kill the Data acquisition process:
  - Press Crtl - Alt – Sup
  - Click on Task manager
  - Right-click on Data acquisition process
  - Select End task
- Switch off the instrument
- Switch back on the instrument
- Restart the ***Data Acquisition software*** (link on the Desktop or in Windows menu).
- Wait the instrument to be ready.

Data acquisition program stops or hangs with sensors clipped on the detector

- Proceed as previously but after restarting the Data acquisition software:
  - When the detector is moving, wait until it reaches a position close to the door
  - Open the door to stop the detector
  - Remove the sensors from the detector by pulling them down manually
  - Close the door
- Proceed to a reset:
  - Select ***Instrument*** → ***Reset***
- Wait the instrument to be ready.

DATA ANALYSIS

Data analysis can be performed on the OctetRED96e computer and with the ***Data Analysis HT 11.1 software***:

- Computer is available anytime when the instrument is free.
- If you need it when a user is running experiments, make sure that him/her will be OK for you to use the computer during his/her free time. NEVER logout an already logged user during experiments.
- Analysis your data during your booking time is better!
- Login to the computer using your IBS/LDAP credentials.

Opening results

- Start the ***Data Analysis HT 11.1 software*** (link on the Desktop or in Windows menu).
- Open your result directory:
    - Click on ***Explore*** in the Icon bar to view data folders, and navigate to find your results directories,
    - Click and drag a data folder to the Experiment builder,

    or
    - Double-click on a data folder,
    - Several data files can be combined into one analysis by dragging additional files. For combined analysis, data must have the same number and name steps and same step durations.

Pre-process data

- Click on ***Pre-processed Data*** in the top tap bar.
- If needed, and in the bottom table, check any data to exclude them from analysis.
- Click on sensors or selected a group of sensors (click & drag or ctrl-click) to display desired sensorgrams.
- Click on the top ***Processed Data Button*** to turn on/off the preprocess display.
- Assign Reference Sensors (no ligand):
    - Navigate to the ***Reference Sensor Tab***,
    - Click on sensors or selected a group of sensors (click & drag or ctrl-click) to display desired sensorgrams,
    - Select the desired sensors by left click on individual sensor, click & drag/ctrl-click on several sensors or on the column number,
    - Right click on the selection,
    - Navigate to ***Set Sensor Type*** and Select ***Reference sensors***. Sensor icons change to diamond,

      Or
    - Click on ***Reference*** icon. Sensor icons change to diamond,
    - Right click on plate map,
    - Navigate to ***Subtract Reference*** and Choose one of the subtraction methods depending on your experimental settings (in Column, in Row, by pattern…). Sensor icons change to void icons,

      or
    - Select all sensors, Navigate to ***Subtract Reference*** and Choose ***Subtract Reference in Selected Sensors***. Sensor icons change to void icons,

- Assign Reference Samples (zero-concentration of analyte):
    - Navigate to ***Reference Sample Tab***,
    - Click on well or selected a group of wells (click & drag or ctrl-click) to display desired sensorgrams,
    - Select the desired wells by left click on individual well, click & drag/ctrl-click on several wells or on the column number,
    - Right click on the selection,
    - Select ***Reference sample.*** Well icon change to diamond,
    - Right click on plate map,
    - Navigate to ***Subtract Reference,*** and Choose one of the subtraction methods depending on your experimental settings (in Column, in Row, by pattern…). Well icons change to void icons,
      or
    - Select all desired sensors, Navigate to ***Subtract Reference*** and Choose ***Subtract Reference in Selected Sensors***. Sensor icons change to void icons,
- Data corrections:
    - Navigate to ***Data correction Tab***,
    - Align Y Axis to align on baseline:
        - Choose one of the ***Align Data to:*** options:
            - *None*,
            - *Average of Baseline Step* (default – 5 seconds before association step),
            - *Start of Association Step,*
            - *Average a segment of Dissociation,*
    - Inter-Step Correction to correct for system artefacts between steps and buffer mismatches:
        - Choose one of the ***Align Data to:*** options:
            - *None* (in case of fast association and dissociation),
            - *Baseline Step* (default – at time 0 second),
            - *Association Step,*
            - *Dissociation Step,*
    - Select ***Savitzky-Golay filtering*** to remove high frequency noise in case of protein/protein interaction or when the signal/noise is high. Never use the filtering with protein/small molecule interaction or when signal/noise is low.

Kinetics analysis

- Click on ***Kinetics*** in the top tap bar,
- If needed, and in the bottom table, check any data to include them from analysis,
- Select in ***Steps to analyze*** panel:
    - *Association,*
    - *Dissociation,*
    - *Association and Dissociation* (default).
- Select in ***Binding mode*** panel:
    - 1*:1*: the classical 1:1 Langmuir model,
    - *2:1 (heterogeneous ligand)*: for a ligand with 2 binding sites, a mixed Ligand or an heterogenous surface,
    - *Mass transfer*: when at the limitation of mass transfer,

    - *1:2 (Bivalent Analyte)*: for an analyte with 2 identical binding sites.
- Select in ***Fitting*** panel:
    - Select in *Type:* ***Global*** (full),
    - Select in *Group by:*
        - ***Sample ID*** (o other grouping scheme) when performing a parallel experiment (Several functionalized biosensors with same ligand density and several analyte concentrations),
        - ***Sensor*** when performing a inline experiment (A single functionalized biosensor and several analyte concentrations with regeneration between cycles),
    - Select in R_{max} *Values* either:
        - **Sensor (unlinked):** when using several functionalized biosensors,
        - **Compound (Unlinked):** when using several analytes,
        - **Linked**: when using a single functionalized biosensor.
- Change **Windows of Interest** if any artefact or bad data have to be removed from the fit (default all data range).
- The fit occurs every time you choose and change any fitting parameters. The fitting curves are in red lines. Examine your fitted curves, residuals, kinetics parameters and fitting statistics to evaluate the validity of it.

Steady-State Analysis

- In case kinetics data reach equilibrium at all analyte concentrations, Steady State analysis can be performed. In ***Steady State*** panel:
    - Select ***Response*** as the mode of analysis,
    - Select Region of Analysis by defining ***Average from X to X***:
        - It should be the equilibrium region for all curves,
        - Default is a 5 second windows before the end of association phase,
    - Navigate to bottom right panel and select ***Steady-State tab*** to view the graph.

For other evaluation tools

Please refer to the OctetRED96e software manual.

Report & Export data

A report can be created and printed with different items from the analysis:

- Click on ***Report*** in the top tap bar,
- Click & Drag any items from the left menu to the page on the right,
- Click on ***Preview printing***,
- In the new window click on ***printer icon***.

Data, graphs and results can be exported either in excel file (.xlsx) or CVS file (.cvs) or text file (.txt):

- To export preprocessed data (CVS file):
    - Click on ***Preprocessed Data*** in the top tap bar,
    - Click on ***Export Raw Data to File*** icon.
- To export processed data (CVS file):
    - Click on ***Preprocessed Data*** in the top tap bar,
    - Click on ***Export Preprocessed Data to File*** icon to export unprocessed data.

- To export a preprocess report (Excel file) with Summary, Data graphs, Tray and plate images and details:
  - Click on ***Preprocessed Data*** in the top tap bar,
  - Click on ***Export Experiment Summary, Preprocessed*** **Data and analysis data to Excel to File** icon.
- To export Fitting curves (Text file):
  - Click on ***Kinetic Analysis*** in the top tap bar,
  - Click on ***Export Fitting Results*** icon.
- To export Fitting Results Table (CVS file):
  - Click on ***Kinetic Analysis*** in the top tap bar,
  - Click on ***Export Table to CVS File*** icon.
- To export a Fitting report (Excel file) with Data graphs, Results tables, Summary, , Tray and plate images and details:
  - Click on ***Kinetic Analysis*** in the top tap bar,
  - Click on ***Export Experiment Summary, Preprocessed*** **Data and analysis data to Excel to File** icon.

A few validation tips

To assess the quality and to validate the fit of your BLI data:

- Magnitude of kinetics constants must be within the instrument specifications:
  - $10^1 < k_a < 10^7 M^{-1}.s^{-1}$
  - $10^{-6} < k_d < 10^{-1} s^{-1}$
- R_{max} must lower than Theorical R_{max} calculated from R_{ligand} and molecular weight ratio.
- k_m (mass transfer kinetics) must be neglectable compared to k_a ($k_m >> k_a$).
- Residuals should be as low as possible and in the same order of magnitude as the instrument noise (0.1 nm).
- Chi^2 (Full X^2) should be as low as possible and in the same order of magnitude as the instrument noise, but it is dependent of number of measurements and of their magnitude. It should be in the range of 0.1 nm^2 per 1 nm of signals.
- R^2 (Full R^2)should be as close as possible of 1: 0.999 is a perfect fit, 0.99 to 0.98 is a good fit, 0.98-0.95 is an average fit, below 0.95 is a bad fit.
- DO NOT surf or shop for kinetics fitting models. More variables in the models always lead to better data fitting statistics. The usage of a model must be motivated; in any doubt use the simplest model.

Other validation tips:

- Validate your experimental settings (quality of samples, immobilization levels, association & dissociation times, concentrations ranges, blank injections...) as suggested in the experimental design section.
- Perform replicates.
- Use different immobilization levels.
- Vary shake speed (for mass transfer limitations).
- Vary contact time to reach equilibrium.
- Check self-consistency; if feasible do kinetics and Steady State analysis.

TO GO FURTHER

OctetRED96e manuals

Instrument and software manuals are available via the software help menu.
Additional application notes are made available to all users. They are electronically stored on the computer via a link found in the following directory: **C(OS):/Octet users/Usefull documentation.**
We recommend to consult the Molecular Devices/Fortébio website for further resources (registration may be needed).

Websites

- ISBG/SPR plateform: www.isbg.fr/biophysics-characterisation/bli/
- Molecular Devices/FortéBIo: https://www.moleculardevices.com/products/biologics

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Collaboration

The collaborative projects (term defined prior experiments) imply that the persons responsible for the platform (Jean-Baptiste Reiser and Anne Chouquet) are co-authors of the published papers.

Service/Autonomous access

Users must mention the platform in any of their publications: "This work used the platforms of the Grenoble Instruct-ERIC Center (ISBG: UMS 3518 CNRS-CEA-UGA-EMBL) with support from FRISBI (ANR-10-INBS-05-02) and GRAL (ANR-10-LABX-49-01) within the Grenoble Partnership for Structural Biology (PSB). Authors acknowledge the SPR platform personal, Jean-Baptiste REISER Ph.D. and Anne Chouquet, for their help and assistance."