Biacore T200
Getting Started Guide

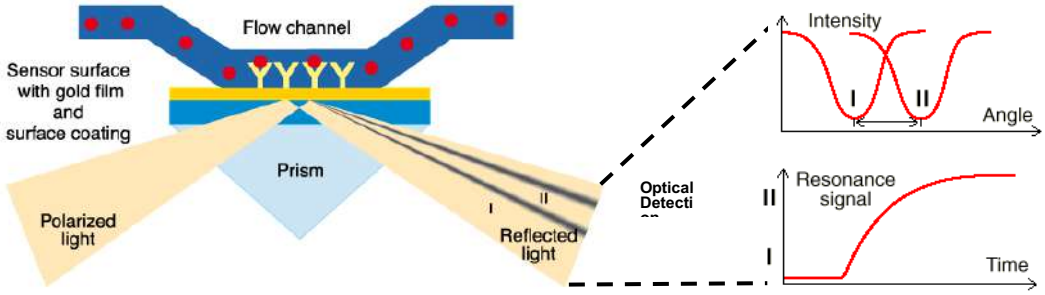
SPR/BLI Platform

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

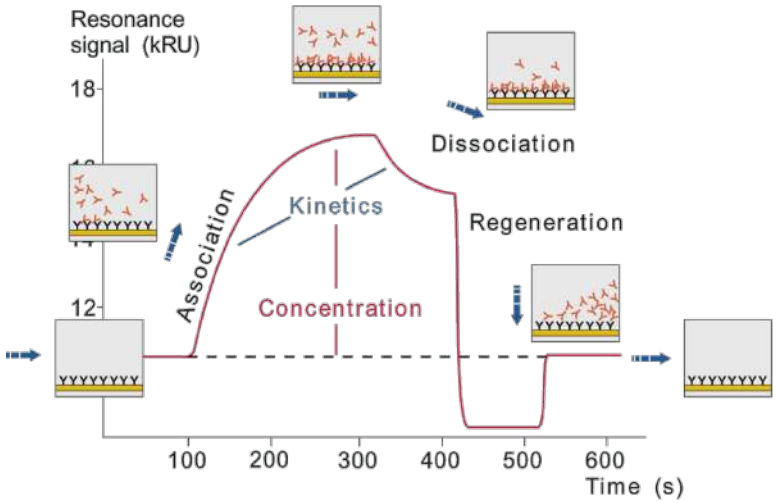
INTRODUCTION

The Biacore T200 is an instrument for Surface Plasmon Resonance (SPR), an optical technique that measures near a metal surface changes in refractive index. Because they are dependent of nature of the constituents at the surface, the SPR technique allows real-time detection and monitoring of biomolecular binding events.

In a typical SPR experiment, one molecule (**Ligand**) is immobilized or captured to a Sensorchip and binding of a second molecule (**Analyte**) is measured under a constant buffer flow. SPR can be then used to measure equilibrium binding constant (affinity, K_D), kinetic binding rate constants (k_a, k_d) and even thermodynamic binding constants (ΔH, $T\Delta S$) without any labelling.



Response is measured in real-time as resonance units or response units (RU) and displayed on a Sensorgram. It is directly proportional to the mass density of biomolecules bound to the surface (1000 RU ⇔ 1 ng/mm² ⇔ 10 mg/ml).



Major steps in a Biacore 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).
- Immobilization or Surface preparation:
  - The Ligand will be directly immobilized or captured via a secondary molecule to the Sensorchip (see further for strategies).
- Interaction analysis:
  - During association phase, the Analyte is injected over the functionalized surface in a constant buffer flow. Contact time and flow rate have to be adjusted (see further for strategies),
  - During dissociation phase, the Analyte is washed out by the constant buffer flow (see further for strategies),
  - Several injections at different concentrations of Analyte is necessary to determine binding constants (see further for methods).
- Regeneration:
  - In order to be reused, the functionalized surface 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 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/spr/) 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 Biacore/GE Healthcare Life Sciences (www.biacore.com).
No other Sensorchips will be accepted for the instrument and they must be Serie S-type Sensorchip.
Users can benefit of the platform discount contract (10% discount). Contact platform personal.

Contacts

Address

SPR/BLI Platform
Institut de Biologie Structurale - UMR 5075 (CNRS-CEA-UJF)
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CS 10090
38044 Grenoble cedex 9
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Platform webpage

http://www.isbg.fr/biophysics-characterisation/spr/

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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 must be prepared in running buffer (same batch or minimum requirement: same composition) as a buffer mismatch will cause optical artifacts. 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 will depend on the flow rate and association/contact time (max volume is 408 µL per injection).
- Analyte samples must be filtered (0.22 µm) or cleared by centrifugation (16 kg, 5-10 min) before experiments.

Running buffer

- Choose a running buffer compatible with Biacore system and in which your samples behave well (PBS, HBS, TBS...).
- Addition of 0.05% Tween-20 is required to prevent non-specific binding.
- DMSO can be used up to 10%.
- Avoid any Glycerol and DTT since they introduce large variation of refracting index.
- 1 L to 2 L of running buffer are required depending of the flow rate for a full week of instrument usage.
- Commercial commonly used buffers can be purchased from Biacore/GE Healthcare Life Sciences (HBS-P+, HBS-EP+, PBS-P+...).
- Buffer need to be filtered (0.22 µm) and contained in glass bottles.

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?

SPR 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...):

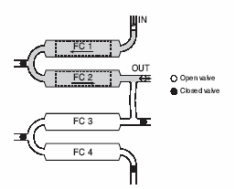
- Direct and irreversible immobilization: Amine coupling (covalent), Thiol coupling (covalent), Aldehyde coupling (covalent), Maleimide coupling (colavent).
- Secondary and irreversible capture: Streptavidin/biotin (fM affinity).
- Secondary and reversible capture: Ni-NTA/His-tag (nM affinity), Antibody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

What sensor Chip?

Biacore/GE sells several types of Sensorchips with different immobilization/capture chemistries.
Biacore T200 requires Series S Sensorchips.
The commonly used are:

- CM5 (Carboxy Methyl Dextran) for amine coupling (irreversible)
  - Maximum target density: 8000-16000 RU,
  - Can be used to create a secondary capture sensor (immobilization of streptavidin, antibody, specific binders...),
  - Alternative CM4 (lower methylation) or CM7 (higher methylation) can be used either to reduce or increased Ligand density,
  - 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),
  - Brute force amine coupling (>1 mg/ml) if standard method doesn't work,
  - Perform a pH Scouting to determine suitable concentration and pH for amine coupling immobilization.
- NTA for His-tagged Ligand captures (reversible):
  - Maximum target density: 1000-5000 RU,
  - 6His-tagged Ligand can be unstable,
  - Capture can be stabilized by extra amine coupling,
  - Easily regenerable with EDTA or EGTA (no feasible if extra coupling).
- SA (Streptavidin) for capture of biotinylated Ligands (irreversible):
  - Maximum target density: 2000-5000 RU,
  - Ligands must be biotinylated (several strategies: Biotin tags, cross-linking...).
- Antibody capture (reversible):
  - Several kits available (His, GST, Fab, Antibody capture kit),

- Homemade capture feasible,
- Easily regenerable with Glycine.



What flow cell?

The Biacore T200 has 4 flow cells (Fc).

- Each experiment uses minimum 2 flow cells.
- Fc1 and Fc3 are used as references (with no immobilized Ligand or dummy Ligand).
- Fc2 and Fc4 would be the functionalized flow cells.

What immobilization level/density?

- For each interaction, calculate a target immobilization level (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 50-150 RU.
- For small molecule Analytes, set R_{ligand} such that R_{max} is about 25.
- 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: 1000 RU = 1ng/mm^2 = 10 mg/ml.
- Low density is always better! It minimizes steric hindrance, aggregation at the surface and mass transport limitation.
- Use Immobilization wizard template with target level in case of amine coupling and in order to fine tune density and to be reproducible.

What reference?

- Use adjacent flow cells as references (Fc1 for Fc2, Fc3 for Fc4).
- A single flow cell reference can be used (Fc1).
- Real time simple reference is performed by the software (Fc2-Fc1, Fc4-Fc3, Fcx-Fc1).
- 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.

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:

- Flowrate.
- Association time (contact time).
- Dissociation time.
- Analyte sample concentrations.
- Regeneration.

What flow rate?

- Recommended flow rate for kinetic assays is minimum 30 µl/min.
- Fast flow rate is important for minimizing mass transport limitation and for good reference subtraction.
- Maximum contact time and maximum flow rate: flow*contact/60=350 s.
- Faster is better!

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 5 min of contact time.
- For small molecule bindings, 30 s to 1 min may be necessary.

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 regeneration conditions?

Proper regeneration conditions have to be found to remove Analyte molecules from the functionalized surface. They consist of injection of 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:
  - Use Biacore/GE regeneration kit,
  - Inject different regeneration buffers in series and during pilot analysis,
  - Test pulses of 30 to 60s,
  - 1 to 2 pulses have to be sufficient,
  - 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; MgCl2 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).
- Perform regeneration scouting manually or with Regeneration Scouting Wizard Template.

What minimum requirement for kinetics assay?

- At least 5 different concentrations of Analyte – best: 8 or more concentrations.
- Spanning a concentration range of 0.1-10 * K_D
- Samples must be diluted in running buffer and in dilution series (1.5 to 3).
- 2 zero-concentrations injections (buffer only) for double reference have to be included, *e.g.* before and after the samples.
- "Startup cycles" prior kinetics analysis are essential for system equilibration (3 to 5 running-buffer injection and regeneration cycles).
- For experiments with DMSO, perform solvent correction in your assays.

What strategy?

Several strategies are available to obtain kinetics and/or affinity:

- The multi cycle kinetics (MCK) is the most common way:
  - Each injection of Analyte or blank is done in separate cycles,
  - Regeneration is performed at the end of each cycle.
- The kinetic titration or Single Cycle Kinetic (SCK):
  - The Analyte injections from low to high concentrations (5) and a long unique dissociation are performed within the unique cycle,
  - Regeneration is then not necessary.
- Steady State analysis:
  - Consist of measuring the response at equilibrium *vs.* Analyte concentration,
  - Same methods as MCK,
  - Can be done with fast-enough dissociating interactions.

What about 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...).
    - Randomizing sample injection is also a way to minimize statistical errors.

WHAT TO BRING FOR YOUR EXPERIMENTS

The minimum requirement is:

- Running buffer(s) in 1 L glass bottles.
- Ligand samples at the highest concentrations (to be diluted in immobilization buffers).
- Analyte samples at the highest concentrations (to be diluted in running buffers).
- Regeneration buffer if known.
- SensorChips: No other Sensorchips will be accepted and they must be Serie S.
- 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 platform makes available the basic and common supplies for experiments on the Biacore T200:

- Biacore T200 instrument.
- 4°C refrigerator and -20°C freezer -20°C for sample storage.
- Bench vortex and centrifuge.
- P20/Tween-20 at 10% stock (added to your running buffers prior experiments – located on the top shelf).
- 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).
- Vials and caps for Biacore T200 (on the bench and 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).
- Filtered water, Desorb, Sanitize, Flow wash and Superclean solutions for instrument maintenance (located in the 4°C refrigerator and on the top shelf).

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: 20 to 30 min.
- pH Scouting prior amine coupling immobilization: 30 to 60 min per ligand sample.
- Amine coupling immobilization: 45 to 60 min per sample.
- Secondary capturing: 20 to 30 min per sample.
- Pilot analyses: ½ day to several days depending on number of injections of samples at different concentrations, test of several contact and dissociations times, of several flow rates and of regeneration conditions.

- MCK/Steady State: 3 to 8 hours or more depending on kinetics and concentrations.
- SCK: 2 to 5 hours depending on kinetics.
- Shutdown sequence: 30 min.

START-UP SEQUENCE

- Before you start, check booking calendar on SPR platform webpage (http://www.isbg.fr/biophysics-characterisation/spr/) and reserve the instrument via the reservation form.
- 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.
- Start the ***Biacore T200 control software*** (link on the Desktop or in Windows menu).
- Check that the waste bottle is empty (right tray).
- Check that the filtered water bottle is filled (right tray).
- Set the Temperatures (default is 25°C):
    - Select ***Tools → Set Temperature…***
    - Wait for the temperatures on the chip and sample compartment to be stable.
- Undock the maintenance chip, have a quick inspection of the sensor square and store it in its dedicated plastic box:
    - Select ***Tools → Eject Chip…***
    - Wait for the Sensorchip compartment door to open automatically,
    - Remove the cartridge from the docking bay,
    - Slide the inner part out from the cartridge,
    - Inspect the plastic sensor square:
        - It should be dry and completely clean,
        - If not, inform the platform personal to proceed a deep cleaning of the instrument,
    - Slide the inner part back in,
    - Store the maintenance chip in its box.
- Dock your Sensorchip:
    - If docking a used Sensorchip, take care to rinse it with water and thoroughly dry the sensor and the cartridge before redocking,
    - Insert the cartridge into the docking bay by following the arrows (inscriptions on top),
    - Close manually the compartment door,
    - In the ***Insert Chip*** window, Select ***New Chip***, and in ***Chip Type*** menu choose the one corresponding to your Sensorchip; or Select ***Reused Chip*** and choose your old Sensorchip Id,
    - Complete ***Id Chip*** field,
    - Select ***Dock Chip***.
- Prepare your running buffer with P20/Tween-20:
    - Add P20/Tween-20 0.05% final directly to your buffer (Stock solution at 10% in the fridge, 5 mL for 1 L).

- Exchange the water bottle connected to line A (left tray) with your running buffer bottle. Make sure the tubing goes all way down the bottle.
- Prime the system with your running buffer:
    - Select ***Tools*** → ***Prime…***
    - Select ***Start***,
    - Specific buffers (with detergents, DMSO, glycerol, high salt content...) need to be primed 2 or 3 times.

RUNNING EXPERIMENTS

Experiments can be run in 3 modes:

Manual run

This mode should not be used for any analysis since the evaluation software will not be able to process these data. It might be used for pilot analysis, binding and regeneration tests and scouting, and indirect captures:

- To start a new manual run:
  - Select ***Run*** → ***Manual Run…*** or click on the ***Start Manual Run*** button,
  - In the new window:
    - Enter a flow rate value (5-10 µl/min for immobilization or capture, >30 µl/min for binding analysis – max 100 µl/min),
    - Select Flow Cell and Flow Path configuration,
    - Select the rack type (*i.e.* ***Sample & Reagent Rack 1***),
    - Select ***Start,***
  - In the next window, follow the software instructions to save the result data file in your subdirectory (*Desktop → Bia Users → Your subdirectory*),
  - New window sections may open (Control, Sensorgram, Keywords, Report points) and SPR signal (Sensorgram) starts to be displayed,
  - Wait 2 to 3 min that the baselines are stable.
- Several buttons are available to control the instrument during manual runs:
  - ***Flow Command***: to change the flowrate (from 5 to 100 µl/min),
  - ***Flow Path Command***: to change Flow cell circuit (among the Fc selected from the start),
  - ***Eject Rack Tray*** (see below),
  - ***Injection command/Stop*** (red syringe): select the vial position and the contact time (total volume needed is indicated),
  - ***Regeneration Injection command/Stop*** (blue syringe): select the vial position and the contact time (total volume needed is indicated),
  - ***New cycle***: to start a new Sensorgram that would be saved in the same result file,
  - ***End manual run***.
- Submenus allow to navigate through Sensorgrams:
  - ***Cycle***,
  - ***Curve***.
- To display only one Sensorgram or one type of Sensorgram or all types:
  - Select ***View*** → ***Show...***
- Scale and title can be modified:
  - Select ***View*** → ***Title…*** or ***Scale…***
- Zoom can be done by left clicking and drag selection. To unzoom, double left-click in the Sensorgram window.
- A reference line can be displayed and drag over the curves:
  - Select ***View*** → ***Reference line*** or click on ***Reference line*** button.
- Report points can be added (useful to add your own labels to your Sensorgram, *i.e.* at each injection):
  - Select ***Report Points*** → ***Add…*** *or* click on ***Report point*** button

    - Follow the instructions and ***Id*** field, select ***time,*** check or not ***Baseline*** (to calculate the zero) and ***Add to all curves in the cycle***,
    - At each injection, automatic report points would be added 5 seconds before injection, before and after the injection (*Baseline_x, Binding_x, Stability_x*),
    - Report points can be modified or deleted by right clicking on them in their window,
- Prepare your samples and reagents in Biacore vials:
    - Remove any air bubbles (by pipetting or thoroughly centrifuging),
    - Check that there is no dust or particle,
    - Always cover vials with Biacore orange caps,
- To eject the rack:
    - Select ***Eject Rack Tray*** button,
    - The sample compartment door would be opened for 1 min. After that time, it will close automatically. DO NOT try to stop it.
    - Press on the button between the rack and the rack support to release it,
    - Slide the rack out of the sample compartment,
- Add your samples and reagents to the rack.
- To insert the rack:
    - Select ***Eject Rack*** button,
    - Slide the rack in the sample compartment and push it until the "click" noise,
    - Close the sample compartment door:
        - DO NOT try to close the door manually, you might broke the door mechanism,
        - Select ***OK***.
- Pursue your experiment as desired by selecting any action.

Wizard Templates

The most commonly used experiments can be run from a Template implemented in the Biacore T200 software package.
The wizard template methods will produce data in a format that is directly read by the Biacore T200 Evaluation software and will make the data processing straightforward.
To start a new or saved template:

- Select ***File*** → ***Open/New Wizard Template…***
- Select a template among the Experimental Categories (for details, consult Biacore manuals):
    - Surface preparation:
        - Immobilization pH Scouting: for testing several pH and Ligand concentrations prior direct immobilization. Use the pH scouting kit to prepare your Ligand sample dilutions.
        - Immobilization: for direct immobilization of the Ligand and reference cells. New method with pre-concentration step and target to reach the right Ligand density and reproducibility. Use this automatic method for any amine coupling immobilization and use the Amine Coupling kit.
    - Assay development:

        - Regeneration Scouting: for testing and determine surface regeneration buffer. Use Regeneration kit,
        - Buffer Scouting: for testing different running buffers,
        - Surface Performance: for testing Ligand stability over regenerations.
    - Control Experiments
        - Kinetics – linked Reactions,
        - Kinetics – Mass Transfer.
    - Assay
        - Kinetics/Affinity: for MCK and Steady State assays,
        - Binding Analysis: for single or in series injections (up to 4) plus regenerations (up to 2). Useful for repeated pilot analyses.
        - Concentration Analysis: for determining Analyte active concentration. Need a standard.
        - Thermodynamics: run MCK at different temperatures to determine thermo parameters.
- Follow the software instructions and design your experiment (see above for more detailed tips).
- The last windows of any wizard templates would give a map to fill the rack:
    - If needed, change the rack type that you will use (*i.e.* ***Sample & Reagent Rack 1***)
    - To automatically populate rack map:
        - Select ***Menu*** → ***Automatic Positioning…*** or ***Default Positions…***
        - Select ***OK***
    - For technical replicates (same samples in same vials), change pooling options:
        - Select ***Menu*** → ***Automatic Positioning…***
        - Select ***Yes*** in ***Pooling*** menu,
        - Select ***OK***.
- Prepare your samples and reagents in Biacore vials:
    - Remove any air bubbles (by pipetting or thoroughly centrifuging),
    - Check that there is no dust or particle,
    - Always cover vials with Biacore orange caps,
    - Always perform dilution series for kinetics analysis,
- To eject the rack:
    - Select ***Eject Rack*** button,
    - The sample compartment door would be opened for 1 min. After that time, it will close automatically. DO NOT try to stop it.
    - Press on the button between the rack and the rack support to release it,
    - Slide the rack out of the sample compartment.
- Add your samples and reagents to the rack according to the map.
- To insert the rack:
    - Select ***Eject Rack*** button,
    - Slide the rack in the sample compartment and push it until the “click” noise,
    - Close the sample compartment door:

        - DO NOT try to close the door manually, you might broke the door mechanism,
        - Select ***OK***.
- Pursue your experiment setup.
- To save a Wizard Template:
    - Select ***Menu*** → ***Save Wizard Template as…***
    - Navigate in subdirectory to select a subdirectory (*Bia Users* → *Methods And Templates*),
    - If needed create a subdirectory to your name,
- To begin the run, in the very last windows:
    - Select ***Start***,
    - Follow the software instructions to save data file in your subdirectory (*Desktop* → *Bia Users* → *Your subdirectory*),

Methods

More complex experiments can be designed with the Method Builder.
The easiest way to create a Method is by modifying an existing method or by converting a Wizard Template to a Method (for details, consult Biacore manuals).
Some specific experiments are predesigned by Biacore:

- The most useful ones are (for details, consult Biacore manuals):
    - Affinity in solution,
    - Calibration-free concentration (CFCA),
    - GST kinetics,
    - LMW kinetics, screen, single-cycle kinetics,
    - Single-cycle kinetics,
- They can be found in ***Biacore Methods*** subdirectory.

To start a Method:

- Select ***File*** → ***Open/New Method…***
- To convert a Wizard Template to a Method
    - Check the box ***Show Importable Wizard Templates***.
- Navigate in the subdirectories, select an existing *Method* or *Wizard Template* and select ***Open***.
- Or Select ***New*** to build a Method from scratch.
- Modify the Method to design your experiment (see above for more detailed tips).
- Select ***Setup Run***.
- Follow the software instructions.
- The last windows of any Methods would give a map to fill the rack:
    - If needed, change the rack type that you will use (*i.e.* ***Sample & Reagent Rack 1***)
    - To automatically populate rack map:
        - Select ***Menu*** → ***Automatic Positioning…*** or ***Default Positions…***
        - Select ***OK***
    - For technical replicates (same samples in same vials), change pooling options:
        - Select ***Menu*** → ***Automatic Positioning…***
        - Select ***Yes*** in ***Pooling*** menu,
        - Select ***OK***.

- Prepare your samples and reagents in Biacore vials:
  - Remove any air bubbles (by pipetting or thoroughly centrifuging),
  - Check that there is no dust or particle,
  - Always cover vials with Biacore orange caps,
  - Always perform dilution series for kinetics analysis,
- To eject the rack:
  - Select ***Eject Rack*** button,
  - The sample compartment door would be opened for 1 min. After that time, it will close automatically. DO NOT try to stop it.
  - Press on the button between the rack and the rack support to release it,
  - Slide the rack out of the sample compartment.
- Add your samples and reagents to the rack according to the map.
- To insert the rack:
  - Select ***Eject Rack*** button,
  - Slide the rack in the sample compartment and push it until the "click" noise,
  - Close the sample compartment door:
    - DO NOT try to close the door manually, you might broke the door mechanism,
    - Select ***OK***.
- Pursue your experiment setup.
- To save a Method:
  - Select ***Menu*** → ***Save Method as…***
  - Navigate in subdirectory to select a subdirectory (*Bia Users* → *Methods And Templates*),
  - If needed create a subdirectory to your name.
- To begin the run, in the very last windows:
  - Select ***Start***,
  - Follow the software instructions to save data files.

SHUTDOWN SEQUENCE

- Undock your Sensorchip:
  - Select ***Tools → Eject Chip…***
  - Wait for the Sensorchip compartment door to open automatically,
  - Remove the cartridge from the docking bay.
- Dock the Maintenance Sensorchip:
  - Insert the cartridge into the docking bay by following the arrows (inscriptions on top),
  - Close manually the compartment door,
  - In the ***Insert Chip*** window, Select ***New Chip***, and in ***Chip Type*** menu choose ***Maintenance Chip***,
  - Select ***Dock Chip***.
- Set the Temperatures back to 25°C:
  - Select ***Tools → Set Temperature…***
  - Wait for the temperatures on the chip and sample compartment to be stable.
- Exchange your running buffer bottle connected to line A (left tray) with a filtered water bottle. Make sure the tubing goes all way down the bottle.
- Check that both filtered water bottles (left and right trays) are at least half-full.
- Empty the Waste bottle (left tray).
- Prime the system with water:
  - Select ***Tools → Prime…***
  - Select ***Start***,
  - If specific buffers (with detergents, DMSO, glycerol, high salt content...) have been used, prime 2 or 3 times with water.
- Run Desorb:
  - Select ***Tools → More Tools → Desorb***
  - Follow software instructions and prepare the Desorb reagents (located on the shelf above the instrument):
    - For protein work, use the standard Desorb reagents: Desorb1 (0.5% SDS) and Desorb2 (50mM Glycine pH 9.5),
    - For small molecule or peptide work with DMSO, replace reagents with: Desorb1 (50% DMSO) and Desorb2 (5% DMSO),
- Complete the instrument logbook (on the top shelve) with your sample and buffer information and details.
- Report any problems to the personal and in the logbook.
- Close the Biacore T200 Control Software and select ***Leave the instrument in Standby mode*** in the last window.
- Logoff from the computer.

DATA SAVING AND ARCHIVING

The SPR platform does not guarantee saving and archiving of user data.
SAVE and ARCHIVE your data (.blr files) and evaluation procedures (.bme files) before leaving the platform.

For internal IBS users

- Login to the computer using your IBS/LDAP credentials or by using generic user local 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 → Bia 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

In case of any trouble with injection, noise, suspected bad Sensorgram due to dirty flow system, clogged IFC…, run in order the following maintenance protocols and check your signal before between each procedure or run a System Check:

Flow System Wash

- Select ***Tools → More Tools → Flow System Wash***.
- Select ***Start***, then ***Next.***
- Unselect ***A: Washing procedure for clogged IFC*** and select ***B: Wash with buffer***.
- Leave all options and follow software instructions to save the result file in Navigate into ***Desktop → Bia Users – Shortcut → GEHC SAV***,
- Check your signal.
- If problem is not solved:
    - Eject your SensorChip and insert the Maintenance Chip
    - Change the buffer for Flow System Wash solution: 50% Isopropanol, 50mM NaOH, 1M NaCl (located in the fridge),
    - Run the Flow System Wash again with:
        - Select A: ***A: Washing procedure for clogged IFC***.
        - Follow software instructions.
- After, the procedure, change the Flow System Wash solution for filtered water and prime with water.
- Eject Maintenance Chip, Insert SensorChip, change the water for your running buffer, prime with your running buffer and check your signal.

Desorb and Sanitize

- Eject your SensorChip and insert the Maintenance Chip.
- Change buffer for filtered water and prime with filtered water.
- Select ***Tools → More Tools → Desorb and Sanitize***.
- Prepare the Desorb and Sanitize reagents (located on the shelf above the instrument and in the fridge):
    - For protein work, use the standard Desorb reagents: Desorb1 (0.5% SDS) and Desorb2 (50mM Glycine pH 9.5),
    - For small molecule or peptide work with DMSO, replace reagents with: Desorb1 (50% DMSO) and Desorb2 (5% DMSO),
    - Sanitize solution:
        - Dilute Biadesinfectant solution or Sodium hypochloride: 6 mL in 80 mL ddH_2O.
- Follow software instructions.
- After the procedure, prime with filtered water.
- Eject Maintenance Chip, Insert SensorChip, change the water for your running buffer, prime with your running buffer and check your signal.

Superclean

- Eject your SensorChip and insert the Maintenance Chip.
- Change buffer for filtered water and prime with filtered water.
- Select ***Tools → More Tools → Superclean***.

- Prepare the Superclean reagents (located in the fridge):
  - Warm filtered water (50°C),
  - 1% Acetic acid,
  - 0.2 M Sodium bicarbonate,
  - 6 M Guanidine (if working with protein) or 50% DMSO (if working with small molecules),
  - 10 mM HCl (if working with protein) or 10% DMSO (if working with small molecules).
- Follow software instructions.
- Prime with water.
- After the procedure, prime with filtered water.
- Eject Maintenance Chip, Insert SensorChip, change the water for your running buffer, prime with your running buffer and check your signal.

System Check

System check can be run instead of checking instrument behavior with your SensorChip and samples. The procedure will check if injections are in the standards of the instrument:

- Eject your SensorChip and insert the System Check Chip (located in the fridge).
- Change buffer for HBS-N buffer and prime with HBS-N buffer (located in the fridge).
- Prepare the BIAtest reagent (15% sucrose - located in the fridge).
- Save data file in ***Desktop → Bia Users – Shortcut → GEHC SAV***
- If check if System Check returns all parameters ***OK***:
  - If yes, eject the System Check Chip, insert your SensorChip, prime with your running buffer and continue your experiment,
  - If not, contact platform personal.

DATA ANALYSIS

Data analysis can be performed on the Biacore T200 computer and with the ***Biacore T200 evaluation 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 or by using generic user local credentials.
- Start the ***Biacore T200 Evaluation software*** (link on the Desktop or in Windows menu).
- Open your result file:
    - Select ***File → Open...***
    - Navigate in the subdirectories to find your own directory, select your result file (.blr – BLR file type) and select ***Open***.
- To add result files or Sensorgrams to an already opened evaluation:
    - Select ***File → Append Result File...***
    - Navigate in the subdirectories to find your own directory, select your result file (.blr – BLR file type) and select ***Open***.
- To save your evaluation procedure:
    - Select ***File → Save...*** or ***Save as...***
    - And follow the instruction to navigate in the subdirectories to find your own directory, and save the file (.bme – BME file type).

Manual display and adjustments

- To display Sensorgams or any plot or evaluation graphs:
    - In the ***Evaluation Explorer***, select any item by left click.
- In the Sensorgrams/plot/graphs windows, several submenus are available to select and display the curves:
    - ***Curve Name***,
    - ***Assay Step purpose***,
    - ***Cycle***,
    - Open the submenu, left click or ctrl+left click to select several curves.
- The submenu ***Tools*** allow you to:
    - Color curves (***Color By***) according to method keywords (Curve, Cycle number, Type, Assay Step and Purpose, Temperature, Buffer, Sample, Ligand, Concentration, Fc...),
    - Align Sensorgrams (***Sensorgramm Adjustement...***):
        - Select ***Sensorgramm Adjustement...***
        - For X-Adjustment:
            - Select either ***Off***, ***Report Point (time=0)*** or ***Injection Event (time=0)***,
            - Select one report or event point you want to adjust on (*i.e. Baseline*).
        - For Y-Adjustment:

        - Select either ***Off***, ***Report Point (response=0)*** or ***Injection Event (response=0)***,
        - Select one report or event point you want to adjust on (*i.e. Baseline*).
    - For Blank subtraction (double reference):
        - Select ***Enable Blank Subtraction***,
        - Select one of your curves with a zero-concentration injection.
    - Select ***OK*** to finish.
- By right clicking in the sensorgram/plot/graphs windows, you can:
    - Change the ***caption*** (title),
    - Change the **scale**,
    - ***Copy Graph***,
    - ***Export curves*** in text format file,
    - Display ***Gridlines***,
    - Change display options of the ***Legend***.

Evaluation

Data created with wizard template methods and methods can be directly processed and evaluated in a semi-automatic manner thanks to implemented tools in the Biacore T200 Evaluation software. After have opened your result files,

- Select one of Evaluation tool corresponding to the Assay purpose of the method that you have performed:
    - Select ***Evaluation → Add...*** or click on one of the following buttons:
        - ***Solvent Correction***,
        - ***Kinetics/Affinity*** for MCK or SCK,
        - ***Concentration Analysis***,
        - ***Thermodynamics***,
        - **Immunogenicity**,
        - ***Screening***,
        - ***Sensorgram Comparison***.

Kinetics/Affinity

Here is a more complete guide that you can follow to evaluate MCK or SCK:

- Select ***Kinetics/Affinity → Surface Bound***
- Select ***Single mode*** or ***Batch mode*** either if you want to evaluate a single or several kinetics experiments
- In the submenus, select:
    - **Curve**,
    - **Ligand**,
    - **Sample**,
    - **Temperature**.
- In the cycle table, select or unselect the curves you want to include or exclude in the evaluation.
- Sensorgrams are colored according to the sample concentrations, and blank (zero-concentration) are in grey.

- In case you want to include several immobilization levels in the same evaluation, select ***Multiple R_{max}*** and add Sensorgrams.
- Select ***Next>***.
- The double reference Sensorgrams (Blank Substracted Sensorgrams) are displayed.
- Select either **Affinity>** or **Kinetics>** button:
    - ***Affinity>*** for Steady State analysis (R_{eq} *vs*. Analyte concentration) and K_D determination:
        - Can be performed only if equilibrium is reached at all concentrations,
        - Select ***Settings*** to adjust the time of calculation of the response,
        - Select ***Next>***,
        - In the Model submenu, select either:
            - Steady State Affinity,
            - Steady State Affinity with Rmax constant,
            - Steady State Affinity Constant Rmax (Multi Site),
        - Select **Fit**,
        - The fitting curve is display as black line and fitted parameters and fitting quality criteria are shown in the ***Report table***:
            - ***K_D***: the affinity parameter,
            - ***R_{max}:*** the maximal response should be lower than theorical R_{max},
            - ***Offset***: should be minimal and close to zero,
            - ***Chi^2***: should be minimal.
        - Select ***Finish***.
    - ***Kinetic>*** for Kinetics analysis (MCK or SCK) and k_a/k_d determination:
        - Select ***Parameters...*** to adjust the fit method and initial values of each parameters if needed,
        - Depending of the interaction model, in the ***Model*** submenu, select either:
            - 1:1 Binding (for a classical 1 to 1 binding),
            - Bivalent Analyte (for an analyte with 2 identical binding sites),
            - Heterogeneous Analyte (for an analyte with 2 different binding sites or a mixed analyte sample),
            - Heterogenous Ligand (for a ligand with 2 binding sites, a mixed Ligand or an heterogenous surface),
            - Two State Reaction (a 1 to 1 binding model with conformational changes),
        - Select ***Fit***,
        - The ***Quality Control*** table displays quality criteria automatically checked:
            - A green check: OK!
            - A yellow exclamation point: check your experiments,
            - A red cross: something wrong with the fit and/or your experiment!

- The fitting curve is displayed as black line and fitted parameters and fitting quality criteria are shown in the Report table:
  - k_a/k_d: the kinetics rates,
  - K_D: the affinity parameter,
  - R_{max}: the maximal response,
  - ***tc*** and k_t: the mass transfert kinetics,
  - ***RI***: the bulk solvent correction,
  - ***U-value***: the uniqueness parameter must as close to one as possible,
  - Chi^2: should be minimal.
- ***Residuals*** table shows the square differences between fitted and measured data.
- Select another model and fit again if necessary,
- Select ***Finish***.

For other evaluation tools

Please refer to the Biacore T200 software manual.

A few validation tips

To assess the quality and to validate the fit of your SPR date:

- Magnitude of kinetics constants must be within the instrument specifications:
  - $10^3 < k_a < 10^7\ M^{-1}.s^{-1}$
  - $5.10^{-6} < k_d < 10^{-1}\ s^{-1}$
- R_{max} must lower than Theorical R_{max} calculated from R_{ligand} and molecular weight ratio.
- k_t (mass transfer kinetics) must be neglectable compared to k_a ($k_t >> k_a$).
- Residuals should be as low as possible and in the same order of magnitude as the instrument noise (1-5 RU).
- Parameter uniqueness or U-Value should be minimum. Since some parameters are linked together and thus their fit correlated, enough data are necessary to leave the underfermentation of the parameter system. The U-value measures the uniqueness of the solution found.
- Chi^2 should be as low as possible and in the same order of magnitude as the instrument noise, but it is dependent of number or measurements and of their magnitude. It should be in the range of 2 RU^2 per 100 RU of signals.
- 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 flow rates (for mass transfer limitations).
- Vary contact time to reach equilibrium.
- Check self-consistency; if feasible do kinetics and Steady State analysis.

TO GO FURTHER

Biacore T200 manuals

Instrument and software manuals are located on the top shelf:

- Biacore Sensor Surface Handbook: an overview of coupling chemistries and immobilization strategies,
- Biacore T200 Getting Started Guide: provides a self-guide tutorial through the basic steps,
- Biacore T200 Instrument Handbook: THE instruction manual for operating the instrument,
- Biacore T200 Software Handbook: THE instruction manual for the Biacore T200 Control and Evaluation software.

Books

- Handbook of Surface Plasmon Resonance – R. Schasfoort & A. Tudis
- Real-time analysis of Biomolecular interaction – K. Nagata & H. Handa
- Surface Plasmon Resonance – Methods and protocols – Serie: Methodes in molecular biology, vol. 627 – N.J. Fischer
- Binding and kinetics for Molecular Biologists – J.A. Goodrich
- Plasmonics: Fundamentals and Applications – S. Maier

Websites

- SPRpages: www.sprpages.nl
- ISBG/SPR plateform: www.isbg.fr/biophysics-characterisation/spr-biacore-technology/
- GE Healthcare/Bicaore: www.biacore.com

Additional software

Scrubber, Evilfit
BiaCalculations
KD-Assitant

AUTHORSHIPS, ACKNOWLEDMENTS

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."