SPR - BiacoreT200

Surface plasmon resonance (SPR)

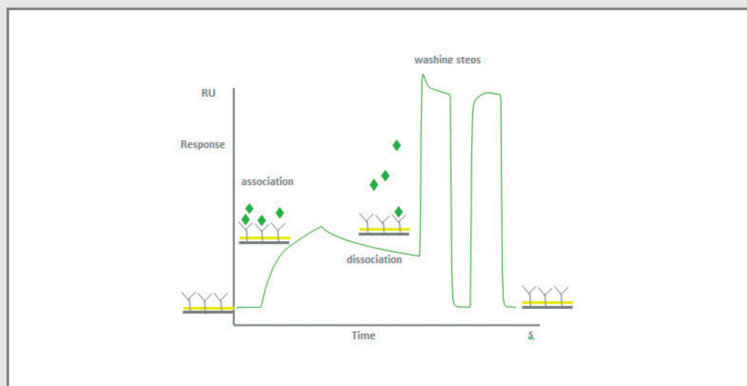
SPR systems exploit the phenomenon of surface plasmon resonance to monitor the interaction between molecules in a real time. One of the interactants is immobilized on the sensor chip surface, while the other is passed over that surface in solution. Applications of SPR include biotherapeutic and drug discovery research, as well as protein activity and stability analysis. SPR is suitable also for characterization of membranes, lipids, nucleic acids and micellar systems. SPR system represents one platform for characterization of biomolecular interactions - kinetics, affinity, specificity, concentration and thermodynamics.

SPR can be used for

- testing of protein activity
- specificity determination - searching for binding partners, characterization of inhibitors affinity, tests for cross-reactivity, eventually directly to test expression of a given protein in cell line cultures
- affinity (kinetics) - kinetic and equilibrium parameters of an interaction, the rates of complex formation (k_a), dissociation (k_d), and equilibrium association/dissociation constants can be determined
- concentration determination - concentration is determined by monitoring the interaction of a molecule with a prepared sensor surface in the presence of a target molecule in solution (solution inhibition) or excess analyte (surface competition)
- multiple interaction during complex formation - complex formation can be monitored as each component is incorporated into a multimolecular complex

Technical Specifications

Instruments: BiacoreT200 (GE Healthcare)



Features:

- versatile, label-free detection system for the study of biomolecular interactions
- analysis of interactants over the broad range of molar masses (from ions to viruses)
- measurement of kinetic constants: k_a in the range of 10^3-$3 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ (proteins) and 10^3-$5 \cdot 10^7$ $M^{-1} \cdot s^{-1}$ (low molecular weight molecules), k_d from 10^{-5}-1 s^{-1}
- due to its high specificity the system is suitable also for small organic compounds (no minimum MW limit), low abundance molecules (concentration >10 pM), rare or sensitive targets, weak interactions (K_D in mM range) and stable binders
- channels: four flow cells that can be used for single, paired or serial runs
- flow cell volume: 0.06 µl, flow cell height: 40 µm
- immobilized interactant consumption: typically 0.03-3 µg/flow cell
- flow rate range : from 1 to 100 µl/min
- baseline noise: typically <0.03 RU (RMS), baseline drift: typically <0.3 RU/min
- injection volume: 2 - 350 µl; sample volume min.: injection volume + 20 to 50 µl (application dependent)
- analysis time per cycle: typically 2 to 15 min
- analysis temperature: 4 - 45°C
- sample tray storage temperature: 4°C to ambient
- integrated buffer degasser, up to four buffers can be tested in a single run
- automation: 48 hours unattended operation
- support of the use of 96- and 384-well microplates, capacity: max. 1 x 96 or 384 well microplate + up to 33 reagent vials

Assemblies:

- sensor chips available - gold layer, hydrophobic layer, NTA for metalo-affinity interaction or carboxymethylated for covalent immobilization of biomolecules

Operational mode:

SPR measurement is performed manually by the user itself after training

Data evaluation software:

BiaEvaluation software (possibility to train people in data processing)

Data collection:

- Direct binding assay - measure the amount of analyte bound directly to the detecting molecule after sample injection
- Binding rate measurement - monitoring of complex formation continuously as a function of time.
- Indirect or competition (inhibition) assays - known amount of detecting molecule is mixed with sample, and the amount of free detecting molecules remaining in the mixture is measured.

Provided services:

- instrument user training
- basic SPR data evaluation training
- consulting/assistance

Sample requirements - importance of sample preparation

- **Sample should be filtrated through 0,2 µm filter as well as a running buffer**.
- Sample environments that differ greatly from the running buffer will give rise to a bulk refractive index (RI) effect that is commonly present during an injection. Bulk refractive index effects do not affect the binding but could hide the interaction. The recommendation is that the samples should be diluted in a running buffer to minimize bulk effects or preferably to use the sample buffer as a running buffer if possible. On-line reference subtraction helps to minimize the effects of bulk.
- sample volume min.: injection volume + 20 to 50 µl (depends on method set-up).
- Most of the buffer compound is possible to use, 70% of ethanol and higher conc. is not allowed.
- On chip immobilization of one interacting partner is essential. Choose wisely the sensor chip that will be used for immobilization of your sample. If you are in doubt, ask for expert consulting on site to minimize the risk of chip degradation.

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

Contacts

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