SPR experiment checklist

Introduction

This document contains a list of steps, which should be checked while performing a surface plasmon experiment. This checklist is for sensor chips with a dextran matrix. In addition, this list can be used for other types of sensor chips. Just skip the items, which are not applicable.

Short List

Pre-immobilization steps

1. Instrument cleaning like Unclog/Flush/Desorb/Sanitize/Other
2. Change to appropriate flow buffer
3. Docking of sensor chip
4. Normalization with 40% glycerol (biacore 2000/3000) or 70% T100/T200
5. Flow of buffer until stable baseline
6. Check detector output
7. Injection of flow buffer / stabilization
8. Injection of analyte / matrix effect

Immobilization

9. Determination of the ligand pre-concentration conditions
10. Immobilization of the ligand
11. Stabilization of the ligand surface
12. Make a reference surface
13. Injection of flow buffer / stabilization

Preliminary experiments

14. Injection of analyte (active surface / association / dissociation)
15. Establishing regeneration conditions
16. Check reference channel for suitability
17. Make calibration plot if necessary
18. Check if Rmax is within the expected range
19. Check for mass transport limitation
20. Check for linked reactions

Experiments

21. Document experimental conditions, questions and results.

Storage of sensor chip

22. Record the storage conditions (buffer composition / temperature)

SPR experiment checklist

Explanation

This checklist is written with the CM5 sensor chip in mind. However, this checklist is also useful with other sensor chips. Just skip the topics, which are not applicable.

Pre-immobilization steps

1. Instrument cleaning
   State which of the procedures are carried out just before starting a new series of experiments.
2. Change to appropriate flow buffer
   State the composition of the run buffer. Buffer must be filtered and degassed. Change buffer with the Prime command
3. Docking of sensor chip
   Record which type of sensor chip is docked. When a previous used chip is docked, refer to the data of the previous immobilization.
4. Normalization with 40% glycerol
   Normalization is done on a new unmodified sensor chip. The 40% glycerol is used to normalize the detector, which will improve sensitivity and reproducibility. Do a Prime to clean the system, otherwise the first injection will not be reproducible.
5. Flow of buffer until stable baseline
   Let the flow buffer run at 25 µl/min until the baseline is constant (dR/dt < 1 RU/min).
6. Check detector output
   Checking the detector output will make sure that the detector and the surface are ok.
7. Injection of flow buffer / stabilization
   Injection of flow buffer over the unmodified surface (all four channels) will give information over the sensor chip surface during injection. In addition, it will give the possible disturbances by the injection system.
8. Injection of analyte / matrix effect
   Injection of the analyte over the unmodified surface (one channel) will give information over the non-specific binding of the analyte to the non-derativized surface. (In case of CM5 the dextran matrix and carboxyl groups.)

Immobilization

9. Determination of ligand pre-concentration conditions
   Ligand pre-concentration conditions are determined by injecting the ligand at different pH and looking at the rate of pre-concentration. High pre-concentration rates are favored but will not always give high ligand immobilization concentrations.
10. Immobilization of the ligand
    Immobilize the ligand using the chemistry, which is suitable for this ligand.
11. Stabilization of the ligand surface
    Some surfaces benefit from a stabilization step with certain chemicals. State the used solutions and the procedure used.
12. Make a reference surface
    Ideally a reference surface will match the protein density and overall nature of the ligand. A non-functional ligand is the best but sometimes hard to get. Using an unmodified or deactivated surface is not a good practice.
13. Injection of flow buffer / stabilization
    Refer to number 7.

Preliminary experiments

14. Injection of analyte (active surface / association / dissociation)
    Injections to establish if the surface is active and get some first impressions of the association and dissociation rate constant. Inject a concentration of 1-10 times the expected KD.
15. Establishing regeneration conditions

The stability of the ligand under regeneration conditions is checked by injecting the analyte and the regeneration solution for three times. The baseline before and after the injection cycle and the Rmax during analyte injection is monitored. The baseline should be the same meaning that the analyte is totally removed and the Rmax should be the same, meaning the ligand is not affected by the regeneration solution.

16. Check reference channel for suitability
    Ideally the reference cell should match the ligand, but without binding the analyte. Inject flow buffer and the buffer for the analyte and observe differences in ligand and reference surface.
17. Calibration plot
    Sometimes a calibration plot is necessary to compensate for unmatched bulk refractive index solutions or differences in behavior of the ligand and the reference channel. State which calibration is done.
18. Check if Rmax is within the expected range
    Rmax says something about the availability of ligand places and the analyte, which is interacting. Check is Rmax is higher or lower than expected. For reaching Rmax during injection an analyte concentration of at least 50 times KD is necessary.
19. Check for mass transport limitation
    Mass transport limitation (MTL) will make the analysis of the sensorgrams more difficult.
    Checking for MTL is done by injecting the analyte at different flow rates. The association and dissociation rate constants should be the same between different flow rates when MTL is not present.
20. Check for linked reactions
    After the initial docking (binding) of the proteins, their orientation and conformation can change resulting in a tighter contact. This process can be observed with longer contact times, which result in an altered (slower) dissociation rate constant.

Experiments

21. Refer to extra experiment documents

Storage of sensor chip

22. The storage of the sensor chip can be important for future uses. State how the chip is stored and which solutions or procedures are used.

References

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2. BIACORE AB; BIACORE Instrument Handbook; 1998
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4. Dorn, I. T., Pawlitschko, K., Pettinger, S. C., and Tampe, R.; Orientation and two-dimensional organization of proteins at chelator lipid interfaces; Biol.Chem.; (379): 1151-1159; 1998
5. Karlsson, R., Fagerstam, L., Nilshans, H., and Persson, B.; Analysis of active antibody concentration. Separation of affinity and concentration parameters; J.Immunol.Methods; (166): 75-84; 1993
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8. Roos, H., Karlsson, R., and Andersson, K.; A calibration routine to improve the interpretation of low signal levels and low affinity interactions.; 1998

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