SPR samples checklist

Introduction

This document contains a list of steps, which should be checked before a surface plasmon experiment is done. This checklist is for sensor chips with a dextran matrix.

Buffer

The buffer used in experiments can make a significant difference in binding of the analyte to the ligand (1, 4). Additions such as detergents, chelating agents or denaturing chemicals have their influence on the binding characteristics and stability.
Starting from scratch, use the standard flow buffer (also for dialysis and sample preparation) but check if the buffer is compatible with your experiments. The standard buffer contains 10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.01% P20. The salt is necessary to reduce non-specific binding, P20 (polyoxyethylene sorbitan) is a non-ionic detergent to avoid adsorption of the analyte to the flow channels, and EDTA will chelate contaminating metal ions. After preparing filter through a 0.22 μm filter and degas before use.

Table: common used buffers in Biacore

Name	Composition
HEPES	10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% P20
PBS	10.1 mM Na2PO4, 1.8 mM KH2PO4 pH 7.4, 137 mM NaCl, 2.7 mM KCl
TBS	50 mM TRIS pH 7.4, 150 mM NaCl

Make enough buffer (2 liter) and filter through a 0.22 μm filter and store in a clean bottle at 4°C.

Sensorchips

Depending on the application an appropriate sensor chip can be chosen.
Please look at the www.SPRpages.nl - sensor chip for the actual available sensor chips.

Ligand

The properties of the ligand, which when known are helpful in experiments, are the molecular mass, iso-electric point and the number of lysine's (2). Information on the binding sites will helpful in the analysis of the sensorgrams. Knowledge about stability under low and high pH and high salt conditions will facilitate the determination of the optimal regeneration conditions.

The ligand should be homogeneous and more than 90% pure when used for covalent binding to the sensor chip. The concentration of the stock solution should be between 0.5 and 2 mg/ml. For one immobilization of a molecule using amine coupling, 1-10 μg is normally sufficient. This amount will depend on the biochemical properties of the molecule to be immobilized, the chosen immobilization chemistry and the applications to be performed (3). The composition of the stock solution should have a salt content below 200 mM and should not contain reactive compounds (e.g. Tris, glycine or BSA with amine coupling).

In the capturing system, the ligand of interest is captured by a specific antibody that is immobilized on the chip. This allows the ligand of interest to be drawn ('purified') from a complex mixture providing the capturing system is sufficiently selective.

Analyte

The properties of the analyte that are helpful in experiments are the molecular mass (should be > 5 kDa for kinetic studies) and pI. The pI should be below nine; otherwise, a lot of non-specific binding is to be expected. Data about binding sites will speed up analysis of sensorgrams.
The analyte concentration of stock solutions should be between 0.1 - 1 mg/ml and free of particles and compounds with large refractive index (e.g. glycerol). The amount needed is about 10 - 100 μg depending on

the K_D (3). The buffer of the analyte should match the flow buffer (dialysis) for precise kinetic measurements. Crude mixtures (such as culture media) can be used for screening purposes.

Possibility of positive and negative controls
Are positive and negative controls available for one or both of the interactants? The use of antibodies to detect protein binding to the chip can be helpful. In addition, a known interacting protein can be used to confirm that the ligand is present and available in its proper conformation.

References

1. **Andersson, K., Areskoug, D., and Hardenborg, E.**; Exploring buffer space for molecular interactions. *J.Mol.Recognit.* **(12)**: 310-315; 1999.
2. **Beattie, J. et al**; Molecular interactions in the insulin-like growth factor (IGF) axis: a surface plasmon resonance (SPR) based biosensor study. *Mol.Cell Biochem.* **(.)**: 2007.
3. **BIACORE AB**; Application areas for Biacore systems. 2000.
4. **Nordin, H. et al**; Kinetic studies of small molecule interactions with protein kinases using biosensor technology. *Anal.Biochem.* **(340)**: 359-368; 2005.

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