Working with liposomes and membrane proteins in Biacore systems

This document presents an overview of current strategies with selected references for working with liposomes and membrane proteins in Biacore systems. Approaches to the problem of maintaining functional integrity of membrane proteins in the hydrophilic environment of a Biacore assay may be broadly divided into:

- Solubilized proteins
- Soluble protein domains and variants
- Membrane-like environments
- Immobilized whole cells

Solubilized proteins

Detergent-solubilization of membrane proteins has proved to be a successful approach in many cases. Both ionic and non-ionic detergents have been used: the choice of detergent may be crucial for retaining protein activity. Generally, detergents should be used at concentrations above their critical micelle concentration (CMC). Micelles formed above the CMC mimic a membrane environment more closely. Whether detergent needs to be included in the running buffer to prevent loss of protein activity varies from case to case.

An alternative to detergents for solubilization is the use of amphipols, which are short soluble polymers carrying numerous hydrophobic side chains. Amphipols constructed with a biotin residue allow capture of amphipol-stabilized membrane proteins on Sensor Chip SA.

References


Karlsson, O (GE) in collaboration with Miller, D (Merck), unpublished. Binding of small molecules to the nuclear membrane protein FLAP.


Soluble protein domains and variants

Soluble extra- or intracellular domains of transmembrane proteins can be studied using standard techniques for working with soluble proteins in Biacore systems. Synthetic peptides may also be constructed to mimic selected functional domains.

There is of course no guarantee that a soluble extracellular domain will exhibit the same binding properties as the intact transmembrane protein. For example, different binding kinetics were observed for Exendin-4 interacting with full-length glucagon-like peptide-1 receptor and the extracellular N-terminal domain; however, β-secretase 1 (BACE1) showed similar binding to full-length receptor and the extracellular domain.

As an alternative to using soluble protein domains, mutant receptors with improved stability in aqueous environments have been exploited in at least one instance (Rich et al, 2011).

References

Membrane-like environments

Membrane-like environments present membrane proteins in a more or less natural environment, surrounded by lipids. The lipids may be natural or synthetic. Distinction is made between three organizational structures of lipids in aqueous environments:

- Micelles have a hydrophilic exterior formed from the lipid “heads” and a hydrophobic core formed from the “tails”. They are essentially monolayer structures and do not support transmembrane proteins. Micelles are seldom used in Biacore studies.
- Liposomes are closed bilayer structures with an aqueous core.
- Bilayer sheets are open structures.

Unchanged illustration taken from Wikipedia http://en.wikipedia.org/wiki/Lipid_bilayer
Several membrane-like environments can be used:

- Natural and synthetic proteoliposomes
- Supported lipid bilayers
- Nanodiscs
- Virus-like lipoparticles

**Proteoliposomes and reconstituted membranes**

Natural proteoliposomes are obtained from disrupted cell membrane preparations derived from cells infected with an expression vector for the target membrane protein. Without recombinant techniques, the levels of target protein in cell membrane preparations are generally too low to be useful in studies using Biacore systems. Specificity may also be an issue since natural membrane preparations inevitably contain a wide range of components.

Depending on the conditions, membranes may attach to the sensor surface as vesicles, liposomes or supported lipid bilayers. These forms are largely equivalent from the viewpoint of accessibility of the membrane proteins. The amount of material attached to the surface gives some indication of the state of the membranes, with liposomes and vesicles generally giving higher responses than supported bilayers.

The use of synthetic liposomes allows more control over the specificity and concentration of liposome components.

Full-length membrane proteins have also been successfully captured on the sensor surface and stabilized by reconstitution of a lipid membrane *in situ* on the sensor chip.

**References**


**Nanodiscs**

Nanodiscs are small self-assembled bilayer discs with a diameter of about 10 nm, prepared from synthetic lipids such as POPC and with their edges sealed by a belt of recombinant membrane scaffold proteins. Nanodiscs with inserted membrane proteins have been used both as ligands and analytes in Biacore studies.

**References**


**Virus-like lipoparticles**

Lipoparticles are created by co-expressing a membrane protein and a retroviral core protein (Gag) in mammalian cells. The Gag core self-assembles and buds from the host cell, carrying with it the membrane protein of interest. References

**References**


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**Cells or cell membranes as ligands**

Using whole cells in Biacore systems to study membrane proteins is technically feasible but must deal with the issues of sensitivity and specificity. For example, with 10 000 RU of a total membrane preparation immobilized on the sensor surface, the expected $R_{\text{max}}$ for binding of neuropeptide Y is less than 1 RU, and a large number of potential binding partners for the analyte are likely to be represented in the membrane preparation. Whole cells or membrane preparations are simply not feasible for work with low molecular analytes.

Expected $R_{\text{max}}$ values for antibodies may however be of the order of 10 to 20 RU, which is readily measurable with Biacore systems today. Unpublished work at GE has shown that Jurkat cells immobilized on Sensor Chip CM5 by aldehyde coupling showed specific binding of mouse anti-human CD4, CD25 and CD247 but not the negative control CD19, and retained the characteristics of intact cells for at least 24 hours. Observed responses were in the range 5 to 10 RU and were sufficient to determine kinetic characteristics of the binding.

Systems such as this may prove useful in antibody screening work.

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**Tips and techniques**

**Ligand or analyte?**

Should you design your assay with the membrane protein attached to the surface (ligand) or injected in solution (analyte)?

Both approaches have been used successfully, and the choice is determined by the same kind of considerations as for Biacore studies using soluble proteins. One aspect in general favor of using captured liposomes as ligands is the ease of regeneration with detergent such as octyl glucoside: scouting for protein-specific regeneration agents is usually not necessary.

**Choice of sensor chip and attachment method**

Solubilized proteins may in principle be immobilized on the sensor surface using any of the standard immobilization techniques for proteins. Choice of method and optimal conditions will vary according to the properties of the protein in question. Capturing tagged or biotinylated membrane proteins on the sensor surface is used more and more as an attachment method, and enhances the specificity of attachment from natural membrane preparations.

Sensor Chip HPA has a flat hydrophobic surface which can be coated with a monolayer of lipids, allowing studies of peripheral membrane proteins.
Liposomes, nanodiscs and other lipoprotein particles may be captured non-specifically by hydrophobic interaction on Sensor Chip L1, or specifically using antibody interaction, a tag or attached residue such as biotin on a hydrophilic surface.

In unpublished screening work done at GE in collaboration with F. Hoffmann-La Roche Ltd, Sensor Chip CM5 was found to exhibit lower levels of “stickiness” for low molecular weight analytes than Sensor Chip L1. This may indicate antibody-mediated capture of liposomes on Sensor Chip CM5 is preferable for applications of this type.

**Choice of detergents**

For detergent solubilization of membrane proteins, the choice of detergent may be important for protein activity. Both ionic and non-ionic detergents are frequently used. The following detergents have been found useful:

- $n$-dodecyl-$\beta$-D-maltoside (DDM)
- cholesteryl hemisuccinate (CHS)
- 3-[[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS)

A “magic mixture” for work with GPCRs (presented by H Heerklotz, Biozentrum, Switzerland at the Biophysical Society in 2007) consists of 0.5% CHAPS, 0.1% DDM, 0.1% CHS in 50 mM Tris-HCl, 200 mM NaCl.

The Membrane Protein Purification Kit available from GE (product code 28-9805-82) provides a convenient source of the following detergents which may be useful for detergent screening work:

- $n$-Dodecyl-$\beta$-D-maltoside (DDM – non-ionic)
- $n$-Decyl-$\beta$-D-maltoside (DM – non-ionic)
- Lauryldimethylamine-N-oxide (LDAO – zwitterionic)
- N-Dodecylphosphocholine (FOS12 – non-ionic)
- Dodecyl octaethyleneglycol ether (C12E8 – non-ionic)
- Cyclohexyl-1-pentyl-$\beta$-D-maltoside (Cyml 5 – zwitterionic)
- $n$-Octyl-$\beta$-D-glucoside (OG – non-ionic)