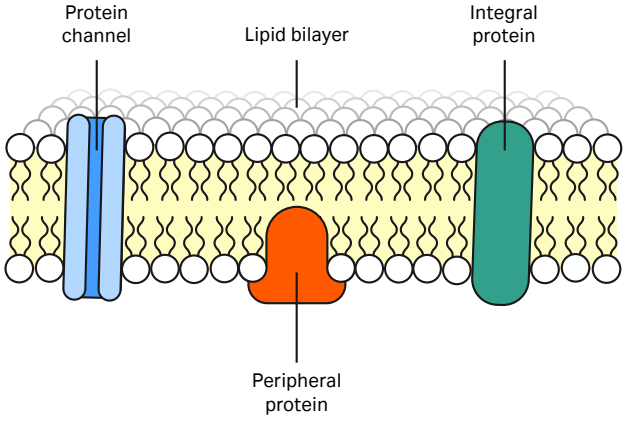
Procedure

Working with liposomes and membrane proteins in Biacore™ systems

Introduction

Membrane proteins are essential to cell function. These proteins send and receive chemical signals, and transport nutrients, ions, and waste across the cell membrane. They are of great biomedical interest — more than 50% of current drug targets are membrane proteins, but studying them can be difficult. Most biophysical techniques were optimized for water-soluble proteins in an aqueous environment, where membrane proteins show poor stability. Fortunately, several tools and techniques are available to help you overcome this obstacle.



Membrane proteins are classified as peripheral or integral.

- Peripheral membrane proteins are present on one side of the membrane. Some are transiently attached and some are water soluble.
- Integral membrane proteins have one or more transmembrane regions that span the membrane. They are insoluble in water.

Here we focus mainly on integral membrane proteins, as they generally present a bigger challenge than peripheral membrane proteins.

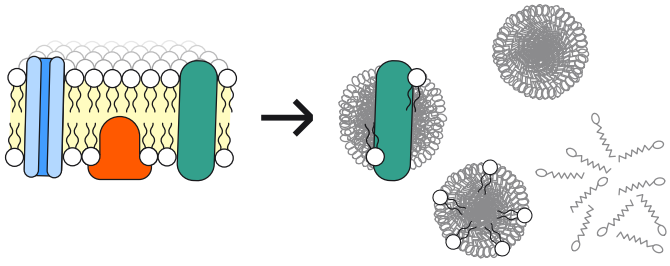
Preparing your membrane protein samples for Biacore™ analysis

We want to give you an overview of current strategies that you can use when preparing liposomes and membrane proteins for Biacore™ analysis. These strategies can broadly be divided into:

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

Solubilized proteins

Membrane proteins can be dispersed in water-based solutions by adding detergents. The detergent solubilizes the biomembrane and forms a soluble complex with the lipids and membrane proteins, ready for analytical studies.



Detergent-solubilization can be harsh and there is a risk of disrupting the protein's biologically active structure. With careful optimization it can be used successfully in many cases. Either an ionic or non-ionic detergent can be used, but be aware that some proteins retain functionality in only one type of detergent. Below are a few examples of detergents that have been found useful:

- n-dodecyl-β-D-maltoside (DDM)
- 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)
- Lauryl maltose neopentyl glycol (LMNG)

Addition of cholesterol analogues such as cholesteryl hemisuccinate (CHS) may also help to retain membrane protein stability and function.

There are several papers describing how to perform detergent screening using Biacore™ SPR system (see below). Once a membrane protein is solubilized it can be captured directly from crude supernatants, membrane extracts or from purified samples using standard Biacore™ sensor chips such as Sensor Chip SA or Sensor Chip NA (for biotinylated proteins), Sensor Chip NTA (for proteins containing His-tags) or using antibodies against epitope tags in the protein (e.g., rho1D4 antibody and C9-tag). The functionality of the membrane protein can then be assessed by monitoring binding of an analyte. Biacore™ SPR system provides more information about membrane protein functionality than standard methods such as SDS-PAGE gels, western blotting, and thermal shift assays, while only using small quantities of material. This helps you identify detergents that preserve binding functionality earlier in the purification process.

Generally, you should use detergents at concentrations above their critical micelle concentration (CMC). This is because micelles formed above the CMC mimic a membrane environment more accurately. You may need to include detergent in the running buffer to prevent loss of protein functionality, but this varies from case to case.

You can also use *amphipols* as an alternative to detergents for solubilization. Amphipols are short soluble polymers with numerous hydrophobic side chains. If you use an amphipol that carries a biotin residue, then you can capture your amphipol-stabilized membrane proteins on Sensor Chip SA.

A "capture -stabilize" approach can also be helpful when working with membrane proteins. Chu et al captured His-tagged CXCR5, a GPCR, on Sensor Chip NTA. They then stabilized the captured receptor by limited crosslinking using diluted amine coupling reagents. The resulting surface displayed full ligand binding functionality in a kinetic assay and could be regenerated many times using an acidic solution.

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Soluble protein domains and variants

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

Keep in mind that there is no guarantee that a soluble extracellular domain will exhibit the same binding properties as the intact transmembrane protein. For example, binding kinetics differs when Exendin-4 interacts with full-length glucagon-like peptide-1 receptor compared to when it interacts with the extracellular N-terminal domain of the receptor. On the other hand, 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 used in at least one instance.

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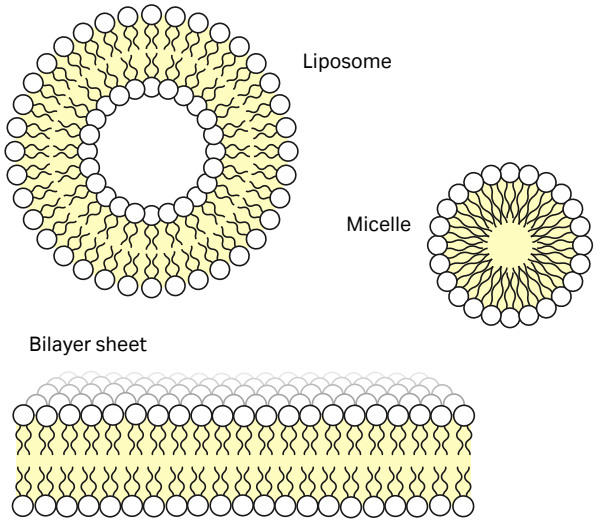
Membrane-like environments

In a membrane-like environment membrane proteins are surrounded by lipids, much in the same way as they would be in the cell membrane. The lipids may be natural or synthetic. In aqueous environments, lipids form three main organizational structures:

- **Micelles** have a hydrophilic exterior formed by the lipid "heads" and a hydrophobic core formed by 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.



You can use several membrane-like environments for your Biacore™ assays, for example:

- Proteoliposomes and reconstituted membranes
- Nanodiscs
- Virus-like lipoparticles (VLPs)
- Styrene Maleic Acid Lipid Particles (SMALPs)
- Salipro® technology

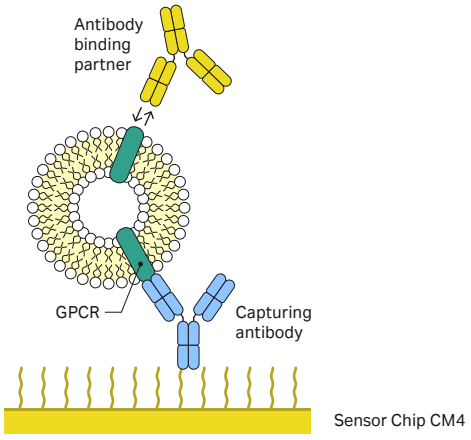
Proteoliposomes and reconstituted membranes

Natural proteoliposomes are obtained from disrupted cell membrane preparations derived from cells infected with an expression vector for the target membrane protein. Recombinant expression is used because the endogenous levels of target protein in cell membrane preparations are generally too low to be useful in Biacore™ studies. Specificity may also be an issue since natural membrane preparations inevitably contain a wide range of components.

Membranes may attach to the sensor surface as vesicles, liposomes or supported lipid bilayers depending on the conditions. Accessibility of the membrane proteins is largely equivalent in all three forms. The amount of material attached to the surface gives you an indication of the state of the membranes; liposomes and vesicles generally give higher responses than supported bilayers.

By using synthetic liposomes, you have 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.



Unpublished work performed in collaboration with one of our customers is illustrated in the figure above. Here, we used antibodies binding to certain epitopes on a G-protein-coupled receptor (GPCR) target. The antibodies were amine-coupled on Sensor Chip CM4. The GPCR target was in a CHO cell membrane. We diluted this membrane 200 times in PBS without detergent and injected over the immobilized antibodies. Next, we injected antibodies binding to other epitopes on the GPCR target and determined their kinetics of binding.

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Nanodiscs

Nanodiscs are small self-assembled bilayer discs with a diameter of about 10 nm, in which membrane proteins can be embedded. The disc edges are sealed by a belt of recombinant membrane scaffold proteins. The disc-like format allows access to protein epitopes on both sides of the lipid bilayer. Nanodiscs can be prepared from one or several synthetic lipids (such as POPC) or from complex lipid mixtures extracted from natural membranes.

Researchers have used nanodiscs with inserted membrane proteins as ligands or as analytes in Biacore™ studies. This suggests that intermembrane interactions where both binding partners are in nanodiscs could also be studied using this strategy. If the nanodisc is used as ligand, affinity tags on the membrane protein or on the scaffolding protein may be used to capture the nanodisc particle on the sensor chip surface. Empty nanodiscs (lacking membrane protein) can be captured on the reference flow cell to control for non-specific binding. Similarly, if the nanodisc particle is used as analyte, it may be a good idea to do control injections of empty nanodiscs over the captured ligand to reveal and potentially correct for any non-specific binding.

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Virus-like lipoparticles (VLPs)

Virus-like lipoparticles (VLPs) are created by co-expressing a membrane protein and a retroviral core protein (Gag) in mammalian cells. As the Gag core self-assembles and buds from the host cell, it carries with it a portion of the cell membrane including the membrane protein of interest. It is possible to introduce tags into the VLP or on the membrane protein itself. The tag can then be used to capture the VLP on a suitable Biacore™ Sensor Chip. For example, Chu et al. captured CD52 in a VLP on Sensor Chip C1 via an anti-CD52 antibody and stabilized the surface via crosslinking with amine coupling reagents.

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Styrene Maleic Acid Lipid Particles (SMALPs)

Poly(styrene-co-maleic acid) (SMA) is an amphiphilic synthetic co-polymer, consisting of alternating hydrophilic maleic acid and hydrophobic styrene groups. SMA polymers can form a belt-like structure that encircles a membrane protein together with a section of its native lipid environment. The resulting disc-like particles are called SMALPs (Styrene Maleic Acid Lipid Particles). SMALPs allow membrane proteins to be studied in their physiological state without any detergent, using many of the biophysical techniques that we use to study soluble proteins. For example, researchers have used SPR to investigate kinetics as well as specificity of antibody binding to integral membrane proteins in SMALPs.

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Salipro® technology

Salipro® is a lipid nanoparticle system based on the saponin protein family, which can stabilize membrane proteins in a lipid environment without detergents. Salipro® particles self-assemble when the membrane protein is mixed with lipids and the Salipro® scaffolding protein. Untagged Salipro® particles can be immobilized onto Sensor Chip L1. Alternatively, you can use a tagged version of the Salipro® scaffolding protein to capture your membrane protein indirectly onto the surface. His Capture Kit (for a His-tagged particle) or Biotin CAPture Kit (for a biotin-tagged particle) have been used successfully.

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Immobilized whole cells or cell membranes

It is possible to use whole cells in Biacore™ systems to study membrane proteins, but maintaining sensitivity and specificity can be a challenge. For example, with 10 000 RU of a total membrane preparation immobilized on the sensor surface, the expected R_{max} for binding of neuropeptide Y is less than 1 RU, and many potential binding partners for the analyte are likely to be represented in the membrane preparation. So, in practice, whole cells or membrane preparations are not suitable for direct binding assays using low molecular weight analytes. However, inhibition assays may be used. In one such experiment, researchers injected whole cells expressing epidermal growth factor receptor (EGFR) over a sensor chip immobilized with the peptide EGF. The detected Biacore™ response was reduced when free EGF peptide was added to the cell suspension at increasing concentrations, confirming the specificity of the interaction.

Expected R_{max} values for antibodies may be of the order of 10 to 20 RU, which is readily measurable with Biacore™ systems today. In an unpublished study, we immobilized Jurkat cells on Sensor Chip CM5 by aldehyde coupling. We saw specific binding of mouse anti-human CD4, CD25 and CD247 but not of the negative control CD19. The Jurkats retained the characteristics of intact cells for at least 24 hours. We observed responses in the range 5 to 10 RU and could determine kinetic characteristics of the binding. Systems such as this may prove useful in antibody screening work.

References

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Assay setup

Running buffer

Running buffer should be chosen based on the characteristics of the ligand and analyte you are working with, the type of assay you plan to run, and the sensor chip used. Cytiva stocks a range of Biacore™ running buffers that provides you with both convenience and quality.

Adding detergent in the running buffer when working with detergent-solubilized proteins may help prevent loss of protein functionality. To avoid sensorgram artifacts, detergent is typically added in a lower concentration in the running buffer than in the buffer used for solubilization. However, this must be tested on a case-by-case basis.

On the other hand, detergent-free running buffer is required for assays in membrane-like environments, as detergents may interfere with the lipid structures. Make sure that your Biacore™ system is clean and free from detergent traces before starting your work, by following the cleaning and maintenance procedures recommended for your instrument.

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. You should make your decision based on the same kind of considerations as for Biacore™ studies using soluble proteins. One advantage of using captured liposomes as ligands is that scouting for protein-specific regeneration agents is usually not necessary. Instead, you can simply use detergents such as octyl glucoside to regenerate the surface.

Choice of sensor chip and attachment method

You can in principle immobilize solubilized proteins using any of the standard immobilization techniques. The specific properties of your protein of interest will determine the best method and the optimal conditions to use.

It is becoming increasingly common to capture tagged or biotinylated membrane proteins on the sensor surface as a method of attachment. This also enhances the specificity of attachment when working with natural membrane preparations. Useful affinity tags when working with membrane proteins include His tag or 1D4 tag.

Sensor Chip HPA has a flat hydrophobic surface which can be coated with a monolayer of lipids. This set-up allows you to study peripheral membrane proteins.

You can capture liposomes, nanodiscs and other lipoprotein particles non-specifically by hydrophobic interaction on Sensor Chip L1, or specifically using antibody interaction, a tag or an attached residue such as biotin on a hydrophilic surface.

In unpublished screening work that we have performed in collaboration with F. Hoffmann-La Roche Ltd, Sensor Chip CM5 exhibited lower levels of "stickiness" for low molecular weight analytes than Sensor Chip L1. Based on this finding, antibody-mediated capture of liposomes on Sensor Chip CM5 may be preferable to non-specific attachment on Sensor Chip L1 for applications of this type.

Run parameters

When selecting run parameters for your assay, the same general rules apply as when working with soluble proteins. Our Application Guides contain helpful information:

https://www.cytivalifesciences.com/en/se/solutions/protein-research/Interaction-analysis-with-Biacore-surface-plasmon-resonance-SPR/Get-started-with-surface-plasmon-resonance-SPR-interaction-analysis#Applicationguideshandbook

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