Chapter 12

Preparation of Lipid Membrane Surfaces for Molecular Interaction Studies by Surface Plasmon Resonance Biosensors

Mojca Podlesnik Beseničar and Gregor Anderluh

Abstract

Surface plasmon resonance has become one of the most important techniques for studying bimolecular interactions. Most of the researchers are using it to study protein–protein interactions, but in recent years membrane model systems have also become available and this makes it possible to study protein–membrane interactions as well. In this review chapter we describe possible ways to prepare lipid membrane surfaces on various sensor chips and some of the experimental considerations one has to take into account when performing such experiments.

Key words: Protein–membrane interactions, lipid membrane, sensor chip L1, sensor chip SA, sensor chip HPA, Biacore.

1. Introduction

Use of optical sensors based on surface plasmon resonance (SPR) has become the most important tool in molecular interaction analysis. More than 1,000 scientific papers were published in 2007 using biosensor platforms from 25 different manufacturers (1). SPR biosensors are used in either qualitative or quantitative mode to determine the binding partners or kinetics and affinity parameters of the interaction, respectively. The use of SPR biosensors is not confined only to basic research laboratories, but is also extensively used in medical diagnostics, monitoring of environmental samples, food analysis, etc. Traditionally, most of the references describe interactions, where both binding partners are proteins, e.g. protein–protein, antigen–antibody,

N.J. de Mol, M.J.E. Fischer (eds.), *Surface Plasmon Resonance*, Methods in Molecular Biology 627,
DOI 10.1007/978-1-60761-670-2_12, © Springer Science+Business Media, LLC 2010

peptide–receptor. Even though lipid membranes are important cell constituents with many associated peripheral and transmembrane proteins, only a small fraction of the rich SPR literature describes interactions of proteins with lipid membranes (1, 2). This is mainly due to difficulties associated with preparations of pure and homogeneous membrane protein samples used for SPR studies. Furthermore, suitable membrane model systems that could be used on optical biosensors became available only recently. In this chapter we provide some examples on the kind of information one can obtain from experiments with SPR biosensors, we give an overview of lipid membrane-mimetic systems that can be prepared and used on Biacore platforms, and we discuss briefly some experimental considerations that are particular for protein–membrane interaction studies. The interested reader will find more information on protein–membrane interaction studies in the following chapters that describe the use of optical biosensors based on SPR in protein–membrane interactions (2), in studies of peripheral membrane proteins that associate with lipid membranes (3–5), membrane-interacting peptides (6, 7), or pore-forming toxins (8).

2. Use of SPR Biosensors in Protein–Membrane Interaction Studies

The SPR literature on interactions of proteins with membranes mainly describes the qualitative and quantitative aspects of peripheral membrane protein interactions with lipid membranes. Typically, one can explore lipid specificity of a peripheral membrane protein by changing the lipid composition of the membrane. Determination of the rate and affinity constants from sensorgrams gives additional information about the interaction of proteins with membranes, especially when the differences between different types of membranes or different variants of proteins are subtle. Often researchers wish to examine effects of particular amino acids on the binding to the membranes (9). Such experiments are relatively easy to perform when the protein attaches reversibly to the membranes. However, most of the membrane proteins are very hydrophobic and may attach non-specifically to the surface of the chip (see below). Sometimes they further aggregate on the surface of the membrane and that often leads to irreversible association with the membranes. A group of peripheral proteins, protein toxins that generate pores in lipid membranes, actually assemble on the surface of the membrane in a multi-step mechanism that involves attachment to the lipid membrane, oligomerization in the plane of the membrane, and irreversible insertion into the membrane (8). It is difficult to interpret the results in such cases and complex binding models should be applied. These

models, however, should be used with caution and it is desirable that some other independent experimental verification of a multi-step mechanism is provided.

Other studies on membrane structure and function have been performed by employing SPR-based biosensors and membrane-mimetic systems. They were, for example, used to study drug–membrane interactions in preclinical drug discovery (10–14), to reconstitute transmembrane proteins on the surface of sensor chips (15, 16), to study the kinetics of removal of particular lipid components from the membrane by either proteins or other molecules (17–19), to characterize intact synaptic vesicles (20), to functionally characterize transmembrane receptors (21, 22). The described studies indicate that SPR-based biosensors can be used in versatile experimental set-ups. For example, varying the membrane model (liposomes vs. bilayer, see below) and composition is one of the basic and yet very informative ways of characterizing the membrane-binding requirements of the protein of interest.

3. Lipid Membrane Model Systems Used in SPR Biosensors

In a typical protein–membrane interaction study the membrane is prepared on the surface of the sensor chip and the protein of interest is injected across such a surface. Although many different approaches on how to prepare a membrane-mimetic surface have been reported, there are only few general methods (*see* **Table 12.1** and **Fig. 12.1**). It is possible to prepare either planar

Table 12.1
Biacore sensor chips that may be used for the preparation of membrane-mimetic surfaces with key references that describe their use

Sensor chip	Description	References
HPA	Formation of stable hybrid bilayer membrane. Only one monolayer is formed on the surface of the chip	(23)
L1	Capture of intact liposomes or other cellular membrane preparations	(37–39)
SA	Capture of liposomes that possess minor amounts of biotin	(42)
CM5	Capture of liposomes by antibodies	(33)
Gold	Capture of liposomes by DNA-derivatized liposomes	(34)

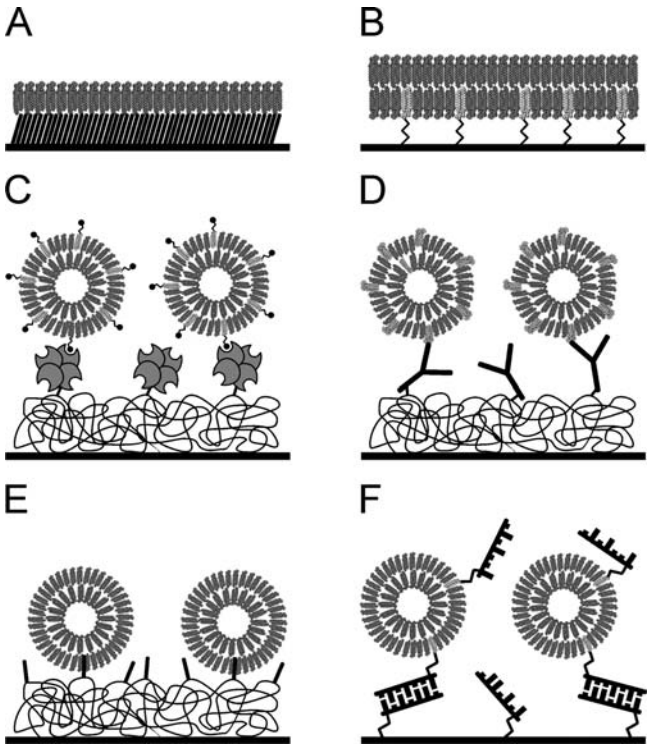

Fig. 12.1. Lipid membrane model systems used in SPR biosensors. (**a**) Hybrid membrane bilayers formed on the surface of hydrophobic layer of alkanes. These can be prepared by using a Biacore HPA sensor chip. (**b**) Tethered membrane bilayers formed on the surface of a gold chip by the use of thiolipids (*light grey*). (**c–f**) Capture of intact liposomes on the surface of sensor chips. (**c**) Capturing may be achieved by the biotin–avidin system, employing low percentage of biotinylated lipids (*light grey*). (**d**) Capture by binding of liposomes to an antibody directed to a lipid component of a membrane bilayer. (**e**) Capture by long lipophilic molecules attached to the dextran matrix (Biacore sensor chip L1). (**f**) Capture by DNA tethers, which allows formation of a 3D liposomal matrix. Reprinted from Beseničar M. et al. (2) with permission from Elsevier.

lipid bilayers or intact liposomes that are captured on the surface of the sensor chips.

3.1. Preparation of Membrane Bilayers

Hybrid bilayer membranes are usually prepared on the surface of alkanethiol self-assembled monolayers, such as Biacore HPA sensor chips (*see* **Fig. 12.1a**). When a solution of small unilamellar liposomes is injected across such a hydrophobic surface, a stable lipid monolayer is formed. Cooper et al. described the general methodology for formation of supported lipid monolayers by using an HPA sensor chip and this study should be taken as a starting point, if one has no experience (23). They compared the monolayer formation when different methods for vesicle preparation, e.g. extrusion or sonication, different lipids, or different buffers were used. Bovine serum albumin was used to check

the quality of the prepared surface. They have also determined the amount of deposited lipids by using ^{3}H-labelled dipalmitoylphosphatidylcholine and found that the correlation of mass of deposited material with the response observed (RU) is the same as for proteins (24). So when a monolayer is formed, fully covering the HPA chip, a response of around 2,200 RU should be expected, corresponding to a mass of 2.0 ng/mm^2 (23). Hybrid bilayer surfaces may be used to study many aspects of protein interactions with membranes, as highlighted by three examples by Cooper et al. (23), but it has some disadvantages that preclude its widespread use. They are hard to work with, as they are very hydrophobic and, more importantly, hybrid bilayers represent only one monolayer of the lipid membrane and, hence, it is not possible to use them to study transmembrane proteins in active form.

There is another model bilayer system encompassing the disadvantages of the hybrid bilayers. A stable membrane bilayer can be tethered on the surface of the gold chip by thiolipids (25–27). The headgroup of these lipids is linked to a thiol group by a long flexible linker. This approach first requires the deposition of thiolipids to form a sparse monolayer. Lipid vesicles are subsequently injected and a continuous bilayer is formed encompassing thiolipids as part of the lipid membrane (*see* **Fig. 12.1b**). In this system both layers of the membrane are fluid and there is an additional aqueous layer between the membrane and the sensor chip. It is possible to include transmembrane proteins in such a membrane (26, 28, 29).

3.2. Capture of Intact Liposomes

Capture of intact liposomes is perhaps the most appealing possibility and a few different approaches were described in the literature. Initial studies involved biotin–avidin interactions, where minor amounts of biotinylated lipid are included in the liposome (30, 31) (*see* **Fig. 12.1c**).

Liposomes can also be retained on the chip by binding to an antibody directed against a specific membrane component, such as lipopolysaccharide (LPS) (32, 33). The paper by MacKenzie and Hirama gives a detailed description of the quantitative analysis of the binding affinity for glycolipid receptors. The authors describe the preparation of liposomes, which include LPS at 1% (w/w) of the total lipid, the deposition of the liposomes on an IgG surface, and the binding of an analyte, a bivalent single-chain Fv (33).

Liposomes may also be captured via DNA tethers; the method requires the use of a membrane lipid attached to a DNA molecule (*see* **Fig. 12.1f**) (34, 35). This approach allows a construction of a 3D surface of liposomes on the surface of the dextran layer. It may find its use in the functional characterization of membrane

proteins embedded in liposomes, as shown recently by Graneli et al. (36). This is particularly appealing, as the transmembrane protein is located away from the sensor chip and at the same time the amount of the protein under study is also increased. Such method is described in **Chapter 16**.

3.3. Capture of Intact Liposomes by the Biacore Sensor Chip L1

By far most of the references in the literature employ sensor chip L1 for studying protein–membrane interactions. This sensor chip possesses lipophilic groups on the surface of the dextran matrix (*see* **Fig. 12.1e**), which efficiently capture intact liposomes. This chip offers some advantages over other described approaches, the most appealing being the easy capturing of liposomes and regeneration of the captured material from the chip, so it may be reused many times. The novice users are directed to papers that describe the deposition of the liposomes on L1 sensor chip and characterization of the so-formed surface (37–39). The advantage of this chip over others is that not only liposomes but also membrane preparations from cell lysates can be used for immobilization. They will be retained by lipophilic anchors and, hence, allow studying membrane binding with biologically more relevant membranes, e.g. binding of pore-forming toxins was studied by using erythrocyte ghosts (40, 41), binding of various drugs to vesicles prepared from intestinal brush-border membranes (13), binding of nanosomes prepared from yeast expressing a mammalian odorant receptor (21).

4. Some Experimental Considerations

Below are some experimental considerations that we found particularly important for experiments on SPR biosensors where membrane systems are used.

4.1. Baseline

There are several reasons for the formation of the drift of the baseline in SPR biosensors, which are extensively covered in instrument manuals. However, when working with hydrophobic surfaces, such as hydrophobic chips (HPA sensor chips) or membranes and liposomes, one should take extra care to minimize all causes that could contribute to the drift formation. One such reason may be the remnants of detergents on the glassware, if common washing services are used. From our own experience, we recommend that all glassware is thoroughly cleaned with copious amounts of distilled water to remove traces of detergents.

Frostell-Karlsson et al. have observed a slight upward drift in the liposome baseline (14). They have systematically checked for the causes of the drift (e.g. buffer composition, lipid membrane

composition, flow rate, liposome injection time, stabilization time after injection, type of regeneration solution used) and found out that the most important parameters for minimizing the baseline drift were a long liposome injection time and additional time used for stabilization of the surface after the liposome injection.

4.2. Referencing

A reference cell is used to subtract the contributions of the buffer to the refractive index and possible unspecific interactions of proteins with the sensor chip. However, hydrophobic molecules may attach to lipophilic anchors on an L1 sensor chip. This is particularly valid for membrane proteins, as they possess extensive hydrophobic surfaces. If it is possible to fully cover the sensor chip, i.e. the sample cell as well as the reference cell, with the lipids, so that no anchors are exposed, and if protein of interest may be prepared in running buffer, so that refractive indexes match, then it is still possible to perform experiments and correct the sensorgrams with the injections of the buffer. However, if the chip surface is only partially covered with lipids, then binding to the lipophilic anchors will be extensive and therefore the flow cell without lipids cannot be used for referencing, as the binding may be even better for the latter. Some other systems that possess a more polar surface should be considered, i.e. a streptavidin sensor chip (SA sensor chip) or an avidin-covered chip, as was used for the characterization of mitochondrial kinases (42, 43).

Another way for referencing is to use a lipid membrane for which the protein of interest has a low affinity. In this way one can account also for non-specific binding of protein to the lipid membrane. Membranes without or with a small percentage of lipid that is recognized by the protein are immobilized on the reference and test flow cell, respectively. Such referencing was used for the protein domains that specifically bind to phosphorylated variants of glycerophosphatidylinositol (5).

4.3. Regeneration

Liposomes captured on the surface of sensor chips are stable for hours. If proteins bind weakly and it is possible to remove them from the liposome surface by buffers with high salt or a change in pH, one can use such surface for many injections with different concentrations of proteins. However, some proteins bind irreversibly and it is impossible to remove them from the membranes. This is particularly true for proteins that insert stably into the membranes. In such cases one should regenerate sensor chips by injections with buffer that contains either detergents or organic solvents to remove all of the lipids from the surface of the sensor chip. Most commonly employed and efficient are solutions of 40 mM *N*-octyl-β-D-glucopyranoside and 100 mM NaOH:isopropanol (2:3; v/v).

5. Conclusions

Optical biosensors are perhaps not so commonly used in membrane biology studies as in some other fields. We believe, however, that a lot of useful qualitative and quantitative information about the interactions of proteins or other molecules with membranes may be obtained. Researchers that study various aspects of membrane biology may benefit tremendously, as highlighted by the cited examples in this chapter. For example, one can look into the membrane-binding properties of the protein of interest without consuming large quantities of the material. Questions can be answered as, does the protein insert into the membrane, or does it bind only superficially? Does a rigid membrane monolayer suffice for binding or does the protein require membrane curvature of the liposomes or at least a more fluid membrane bilayer? What are the pH and salt requirements for the stable binding? How can one prevent binding and the membranolytic activity (if there is one)? All these and many more questions can be answered in quite a short time and with low amounts of protein.

Acknowledgments

We would like to thank the Slovenian Research Agency for support and Vesna Hodnik for critically reading the manuscript.

References

1. Rich, R. L., and Myszka, D. G. (2008) Survey of the year 2007 commercial optical biosensor literature. *J. Mol. Recognit.* **21**, 355–400.
2. Beseničar, M., Maček, P., Lakey, J. H., and Anderluh, G. (2006) Surface plasmon resonance in protein-membrane interactions. *Chem. Phys. Lipids* **141**, 169–78.
3. Cho, W., Bittova, L., and Stahelin, R. V. (2001) Membrane binding assays for peripheral proteins. *Anal. Biochem.* **296**, 153–61.
4. Cooper, M. A. (2004) Advances in membrane receptor screening and analysis. *J. Mol. Recognit.* **17**, 286–315.
5. Narayan, K., and Lemmon, M. A. (2006) Determining selectivity of phosphoinositide-binding domains. *Methods* **39**, 122–33.
6. Mozsolits, H., and Aguilar, M. I. (2002) Surface plasmon resonance spectroscopy: an emerging tool for the study of peptide-membrane interactions. *Biopolymers* **66**, 3–18.
7. Mozsolits, H., Thomas, W. G., and Aguilar, M. I. (2003) Surface plasmon resonance spectroscopy in the study of membrane-mediated cell signalling. *J. Pept. Sci.* **9**, 77–89.
8. Anderluh, G., Maček, P., and Lakey, J. H. (2003) Peeking into a secret world of pore-forming toxins: membrane binding processes studied by surface plasmon resonance. *Toxicon* **42**, 225–28.
9. Stahelin, R. V., and Cho, W. (2001) Differential roles of ionic, aliphatic, and aromatic residues in membrane-protein interactions: a

surface plasmon resonance study on phospholipases A_2. *Biochemistry* **40**, 4672–78.
10. Danelian, E., Karlen, A., Karlsson, R., Winiwarter, S., Hansson, A., Lofas, S., Lennernas, H., and Hamalainen, M. D. (2000) SPR biosensor studies of the direct interaction between 27 drugs and a liposome surface: correlation with fraction absorbed in humans. *J. Med. Chem.* **43**, 2083–86.
11. Baird, C. L., Courtenay, E. S., and Myszka, D. G. (2002) Surface plasmon resonance characterization of drug/liposome interactions. *Anal. Biochem.* **310**, 93–99.
12. Abdiche, Y. N., and Myszka, D. G. (2004) Probing the mechanism of drug/lipid membrane interactions using Biacore. *Anal. Biochem.* **328**, 233–43.
13. Kim, K., Cho, S., Park, J. H., Byun, Y., Chung, H., Kwon, I. C., and Jeong, S. Y. (2004) Surface plasmon resonance studies of the direct interaction between a drug/intestinal brush border membrane. *Pharm. Res.* **21**, 1233–39.
14. Frostell-Karlsson, A., Widegren, H., Green, C. E., Hamalainen, M. D., Westerlund, L., Karlsson, R., Fenner, K., and van de Waterbeemd, H. (2005) Biosensor analysis of the interaction between drug compounds and liposomes of different properties; a two-dimensional characterization tool for estimation of membrane absorption. *J. Pharm. Sci.* **94**, 25–37.
15. Karlsson, O. P., and Lofas, S. (2002) Flow-mediated on-surface reconstitution of G-protein coupled receptors for applications in surface plasmon resonance biosensors. *Anal. Biochem.* **300**, 132–38.
16. Stenlund, P., Babcock, G. J., Sodroski, J., and Myszka, D. G. (2003) Capture and reconstitution of G protein-coupled receptors on a biosensor surface. *Anal. Biochem.* **316**, 243–50.
17. Locatelli-Hoops, S., Remmel, N., Klingenstein, R., Breiden, B., Rossocha, M., Schoeniger, M., Koenigs, C., Saenger, W., and Sandhoff, K. (2006) Saposin A mobilizes lipids from low cholesterol and high bis(monoacylglycerol)phosphate-containing membranes: patient variant Saposin A lacks lipid extraction capacity. *J. Biol. Chem.* **281**, 32451–60.
18. Remmel, N., Locatelli-Hoops, S., Breiden, B., Schwarzmann, G., and Sandhoff, K. (2007) Saposin B mobilizes lipids from cholesterol-poor and bis(monoacylglycero)phosphate-rich membranes at acidic pH. Unglycosylated patient variant saposin B lacks lipid-extraction capacity. *FEBS J.* **274**, 3405–20.
19. Beseničar Podlesnik, M., Bavdek, A., Kladnik, A., Maček, P., and Anderluh, G. (2008) Kinetics of cholesterol extraction from lipid membranes by methyl-[beta]-cyclodextrin – A surface plasmon resonance approach. *Biochim. Biophys. Acta* **1778**, 175–84.
20. Ferracci, G., Seagar, M., Joël, C., Miquelis, R., and Léveque, C. (2004) Real time analysis of intact organelles using surface plasmon resonance. *Anal. Biochem.* **334**, 367–75.
21. Vidic, J. M., Grosclaude, J., Persuy, M. A., Aioun, J., Salesse, R., and Pajot-Augy, E. (2006) Quantitative assessment of olfactory receptors activity in immobilized nanosomes: a novel concept for bioelectronic nose. *Lab Chip.* **6**, 1026–32.
22. Vidic, J., Grosclaude, J., Monnerie, R., Persuy, M. A., Badonnel, K., Baly, C., Caillol, M., Briand, L., Salesse, R., and Pajot-Augy, E. (2008) On a chip demonstration of a functional role for Odorant Binding Protein in the preservation of olfactory receptor activity at high odorant concentration. *Lab Chip.* **8**, 678–88.
23. Cooper, M. A., Try, A. C., Carroll, J., Ellar, D. J., and Williams, D. H. (1998) Surface plasmon resonance analysis at a supported lipid monolayer. *Biochim. Biophys. Acta* **1373**, 101–11.
24. Stenberg, E., Persson, B., Roos, H., and Urbaniczky, C. (1990) Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins. *J. Colloid Interf. Sci.* **143**, 513–26.
25. Lang, H., Duschl, C., and Vogel, H. (1994) A new class of thiolipids for the attachment of lipid bilayers on gold surfaces. *Langmuir* **10**, 197–210.
26. Stora, T., Lakey, J. H., and Vogel, H. (1999) Ion-channel gating in transmembrane receptor proteins: functional activity in tethered lipid membranes. *Angew. Chem. Int. Ed.* **38**, 389–91.
27. Hong, Q., Gutiérrez-Aguirre, I., Barlič, A., Malovrh, P., Kristan, K., Podlesek, Z., Maček, P., Turk, D., González-Mañas, J. M., Lakey, J. H., and Anderluh, G. (2002) Two-step membrane binding by Equinatoxin II, a pore-forming toxin from the sea anemone, involves an exposed aromatic cluster and a flexible helix. *J. Biol. Chem.* **277**, 41916–24.
28. Heyse, S., Ernst, O. P., Dienes, Z., Hofmann, K. P., and Vogel, H. (1998) Incorporation of rhodopsin in laterally structured supported membranes: observation of transducin activation with spatially and time-resolved

surface plasmon resonance. *Biochemistry* **37**, 507–22.
29. Schmidt, E. K., Liebermann, T., Kreiter, M., Jonczyk, A., Naumann, R., Offenhausser, A., Neumann, E., Kukol, A., Maelicke, A., and Knoll, W. (1998) Incorporation of the acetylcholine receptor dimer from Torpedo californica in a peptide supported lipid membrane investigated by surface plasmon and fluorescence spectroscopy. *Biosens. Bioelectron.* **13**, 585–91.
30. Masson, L., Mazza, A., and Brousseau, R. (1994) Stable immobilization of lipid vesicles for kinetic studies using surface plasmon resonance. *Anal. Biochem.* **218**, 405–12.
31. Stachowiak, O., Dolder, M., and Wallimann, T. (1996) Membrane-binding and lipid vesicle cross-linking kinetics of the mitochondrial creatine kinase octamer. *Biochemistry* **35**, 15522–28.
32. MacKenzie, C. R., Hirama, T., Lee, K. K., Altman, E., and Young, N. M. (1997) Quantitative analysis of bacterial toxin affinity and specificity for glycolipid receptors by surface plasmon resonance. *J. Biol. Chem.* **272**, 5533–38.
33. MacKenzie, C. R., and Hirama, T. (2000) Quantitative analyses of binding affinity and specificity for glycolipid receptors by surface plasmon resonance. *Methods Enzymol.* **312**, 205–16.
34. Graneli, A., Edvardsson, M., and Hook, F. (2005) DNA-Based Formation of a supported, three-dimensional lipid vesicle matrix probed by QCM-D and SPR. *ChemPhysChem.* **5**, 729–33.
35. Wikstrom, A., and Deinum, J. (2007) Probing the interaction of coagulation factors with phospholipid vesicle surfaces by surface plasma resonance. *Anal. Biochem.* **362**, 98–107.
36. Graneli, A., Benkoski, J. J., and Hook, F. (2007) Characterization of a proton pumping transmembrane protein incorporated into a supported three-dimensional matrix of proteoliposomes. *Anal. Biochem.* **367**, 87–94.
37. Cooper, M. A., Hansson, A., Lofas, S., and Williams, D. H. (2000) A vesicle capture sensor chip for kinetic analysis of interactions with membrane-bound receptors. *Anal. Biochem.* **277**, 196–205.
38. Erb, E. M., Chen, X., Allen, S., Roberts, C. J., Tendler, S. J., Davies, M. C., and Forsen, S. (2000) Characterization of the surfaces generated by liposome binding to the modified dextran matrix of a surface plasmon resonance sensor chip. *Anal. Biochem.* **280**, 29–35.
39. Anderluh, G., Beseničar, M., Kladnik, A., Lakey, J. H., and Maček, P. (2005) Properties of nonfused liposomes immobilized on an L1 Biacore chip and their permeabilization by a eukaryotic pore-forming toxin. *Anal. Biochem.* **344**, 43–52.
40. Bavdek, A., Gekara, N. O., Priselac, D., Gutiérrez-Aguirre, I., Darji, A., Chakraborty, T., Maček, P., Lakey, J. H., Weiss, S., and Anderluh, G. (2007) Sterol and pH interdependence in the binding, oligomerization, and pore formation of Listeriolysin O. *Biochemistry* **46**, 4425–37.
41. Bakrač, B., Gutiérrez-Aguirre, I., Podlesek, Z., Sonnen, A. F., Gilbert, R. J., Maček, P., Lakey, J. H., and Anderluh, G. (2008) Molecular determinants of sphingomyelin specificity of a eukaryotic pore-forming toxin. *J. Biol. Chem.* **283**, 18665–77.
42. Schlattner, U., and Wallimann, T. (2000) A quantitative approach to membrane binding of human ubiquitous mitochondrial creatine kinase using surface plasmon resonance. *J. Bioenerg. Biomembr.* **32**, 123–31.
43. Schlattner, U., and Wallimann, T. (2000) Octamers of mitochondrial creatine kinase isoenzymes differ in stability and membrane binding. *J. Biol. Chem.* **275**, 17314–20.