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Neutron reflection from interfaces with biological and biomimetic materials

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Abstract

During the past decade, neutron reflectivity has increasingly become an important technique for the characterization of biological and biomimetic thin films. Advancements in instrumentation, sample environment and measurement protocols now make it possible to obtain Angstrom-level information about the composition of these materials along the axis perpendicular to the plane of the membrane. A newly developed phase-sensitive neutron reflectivity technique now allows direct inversion of the reflectivity data to obtain unique compositional depth profiles of the films. This review concentrates on the development of these neutron reflectivity methods for the study of peptide and protein interactions with model membranes. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Neutron reflectivity; Model membranes; Lipid bilayers; Lipid monolayers; Interfaces; Self-assembly

1. Introduction

All cells are enclosed by a biological membrane that defines its boundaries and regulates its interactions with the environment. The biological membrane consists of assemblies of lipid and protein molecules. The lipid molecules form a continuous double layer, or bilayer, which acts as a barrier to water-soluble molecules and provides the framework for the incorporation of the protein molecules. Specialized proteins embedded in lipid bilayers participate in fusion events between cells (i.e. triggered by viruses), regulate ion transport through pores and channels (i.e. neural activities), engage in enzymatic activity at membrane surfaces, and play a role in biological signaling (i.e. receptor proteins activated by hormones).

Cell membranes are sufficiently complicated that they cannot be duplicated in the laboratory for study. Thus, model biological, or biomimetic membranes, which are simpler than cell membranes but mimic their structure and function, are used to study these complicated systems. These systems can consist of lipid monolayers at the air/water interface or planar lipid bilayer systems supported on substrates at the solid/water interface. In both cases, the model systems must be capable of interacting with membrane peptides and proteins. The development of measurement tools for probing the structure and function of these engineered membrane mimics and the cell membrane components incorporated into them is essential for the optimization of their biomimetic character. Over the past decade, the neutron reflectivity technique has been increasingly used to assist in the structural characterization of biomimetic membranes that are in contact with water.

The neutron reflectivity technique has been thoroughly described in the context of the characterization of condensed matter thin film systems at inter-

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faces [1–4]. The specular reflection of neutrons, $R(Q)$, in which the angle of reflection is equal to the angle of incidence, is defined as the intensity of the specularly-reflected beam divided by the intensity of the incident beam. Here, $Q = (4\pi/\lambda)\sin\theta$, where λ is the incident neutron wavelength and θ is the angle of incidence relative to the plane of the film. The measured reflectivity, $R(Q)$, is given by $|r(Q)|^2$, where $r(Q)$ is the complex reflection amplitude. Thus, the phase information is lost in a typical measurement of $R(Q)$.

The neutron scattering length density (SLD) profile of a film, often represented by $\rho(z)$, is a function of film depth, z. For a given layer defined over a range of z values, the SLD is defined as the sum of the coherent neutron scattering lengths of all of the atoms in that layer, divided by the volume of the layer. Thus, the SLD profile is a continuous function of z that relates directly to the chemical composition of the film. For this reason, it is sometimes termed a compositional depth profile.

While $r(Q)$, and thus $R(Q)$, can be calculated exactly from the SLD profile of a film, the SLD profile cannot be obtained directly from the measured $R(Q)$. Thus, data fitting methods are usually used to obtain SLD profiles that are consistent with the reflectivity data. Both standard model-dependent fitting methods, using stepped functions based on the theoretical lipid composition, and model-independent fitting methods, using randomly generated smooth functions represented, for example, by parametric B-splines [5], are used for this purpose.

An advantage in using neutron reflectivity for the study of biological systems is that neutrons are sensitive to the light elements, such as carbon, hydrogen, nitrogen and oxygen, which are so important in these materials. Furthermore, neutrons are sensitive to isotopic differences in these elements. Thus, a simple substitution of deuterium for hydrogen can substantially alter the SLD profiles of biological films, while having minimal effect on their biochemistry. By making several reflectivity measurements on the same system, but with different components deuterated, a more accurate model structure can be determined, since it must be consistent with all of the reflectivity data sets simultaneously. This 'contrast variation' method is used widely in the characterization of biomimetic films and its importance would be difficult to overstate.

This article covers the early neutron reflectivity studies of lipid monolayers and bilayers and describes subsequent efforts to improve the measurement technique in order to measure Angstrom-level details within the structures. The remainder of the paper then concentrates on the study of peptide/protein interactions with biomimetic membrane systems using neutron reflectivity. Finally, a newly developed phase-sensitive neutron reflectivity technique which allows the measurement of the reflection amplitude directly is described.

2. Early neutron reflectivity measurements of lipid monolayers and bilayers

In the early 1990s, the first specular neutron reflectivity experiments were performed on hydrated lipid monolayers and bilayers. The sensitivity of neutrons to hydrogen and the use of contrast variation gave the neutron reflectivity technique an advantage over X-ray reflectivity, especially in the quantification of the hydration of the lipid headgroups. One such experiment [6•] involved the measurement of mixed chain-deuterated dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) monolayers at the air/water interface as a function of lipid phase. The lipid mixture was measured in both D_2O and in a mixture of H_2O/D_2O such that the SLD matched that of the air. The results indicated that a substantial change in lipid headgroup structure accompanied the well-known change in the acyl chain region structure that occurs when the lipids undergo a phase transition from the condensed (gel) state to the expanded (liquid crystalline) fluid state. It was concluded that the carboxyl bonds of the glycerol backbone are hydrated in the fluid state, a finding widely accepted today.

Another early work [7] on lipid monolayers at the air/water interface showed that X-ray and neutron reflectivity used together is another form of contrast variation that can be used to obtain a detailed picture of the interface structure. Both neutron and X-ray reflectivity measurements were performed on dipalmitoylphosphatidylcholine (DPPC) monolayers in the gel state at the air/water interface. Chain-deuterated lipid was used for the neutron reflectivity measurements, at lateral pressures at which isotope effects are negligible, and the neutron reflectivities were measured from the monolayer in contact with water of different isotopic compositions. The resultant model structure, which fit both the neutron and X-ray data, showed that the thickness of the headgroup and number of water molecules associated with the region are both larger than those values obtained for multibilayers. A similar result was later found for supported DPPC bilayers [8] in contact with aqueous solution.

The first neutron reflectivity study of a single lipid bilayer in aqueous solution [9•] was performed on chain-protonated and chain-deuterated DMPC bilayers adsorbed from vesicles onto a planar quartz substrate. The measurements were made at two different

temperatures in order to measure the bilayer above and below the main phase transition temperature of the lipid. Solutions of different SLD were used in order to measure the bilayers at different contrasts. The resultant model structure satisfied all of the data.

Interestingly, the results showed that a 30 ± 10 Å water layer separated the bilayer from the silicon substrate, independent of the lipid phase. Structural details obtained for the bilayer in both phases agreed with those found for vesicles and multibilayers. The use of a chain-deuterated and chain-protonated DMPC bilayer, mixed so that the SLD of the acyl chains matched that of the quartz, allowed for an independent determination of the lipid headgroup structural parameters.

Subsequent to these early experiments, efforts were made to improve the resolution of the neutron reflectivity technique, and thus increase the sensitivity of the measurements to structural details on the order of a few Angstroms. Using a novel experimental setup [10•], reflectivities out to $Q = 0.25$ Å^{-1} were measured from a model biological membrane containing components from the outer membrane of *Neurospora crassa* mitochondria. This represented an improvement of a factor of 2 in Q-range from the earliest lipid bilayer experiment [9•].

This new experimental setup was used to study DPPC phospholipid bilayers adsorbed onto planar silicon substrates from vesicles in solution [8]. Both chain-deuterated and chain-protonated lipids were used and the kinetics of vesicle adsorption were monitored by recording the reflectivity at a fixed Q value. A water layer was found between the silicon substrate and the lipid bilayer, as in the previous lipid bilayer experiment [9•]. Structural parameters agreed with those found in the literature for multibilayer systems, except that the lipid headgroup region in the single bilayer appears to be slightly thicker, and more hydrated, than found in the multibilayer experiments, in support of the early result on DPPC monolayers at the air/water interface [7].

Recently, robust, stable and reproducible double bilayers have been deposited at the solid/liquid interface [11••], providing a promising scaffold for the study of membrane peptide/lipid interactions in a more biomimetic environment. Both DPPC and distearylphosphatidylcholine (DSPC) double bilayers were deposited on silicon (111) surfaces, using a combination of the Langmuir–Blodgett and Langmuir–Schaeffer techniques [12]. The second bilayer was found to be floating freely 20–30 Å above the first. This lipid arrangement is particularly interesting since it may be able to support transmembrane proteins which do not have large extramembrane domains protruding below the upper bilayer.

3. Peptide/protein interactions with lipid monolayers at the air/water interface

Early experiments of peptide interactions with lipid monolayers involved the study of streptavidin binding to biotinylated lipid monolayers at the air/water interface [13–15]. The lipid monolayers at the air/water interface were either biotinylated dipalmitoylphosphatidylethanolamin (biotin-DPPE) or mixtures of biotin-DPPE and DMPC. The protein layer thickness, area per protein molecule and number of water molecules per protein molecule could be quantified on a molecular length scale using the neutron reflectivity technique. The last in this early series of experiments [15] studied the formation of protein layers as a function of surface chemistry of the lipid monolayer.

A more recent experiment used neutron reflectivity to study the adsorption of an actin-binding protein, hisactophilin, to lipid monolayers at the air/water interface [16••] at high lateral pressure. Pure monolayers of DMPC, as well as 1:1 mixtures of DMPC and the anionic lipid, DMPG, were used to form the monolayer. Fully (chain and headgroup)-deuterated DMPC and chain-deuterated DMPG provided contrast between the lipid, protein and water. Different solvent mixtures were used to probe the location of native hisactophilin (DIC-HIS) and a fatty-acid-deficient mutant (EC-HIS). It was found that the proteins bound to the monolayer surface in the absence of DMPG, but the charged lipids strongly favor protein binding. On the other hand, the hydrophobic chains present in the DIC-HIS protein were found to be essential for tight binding of the protein to the monolayer in its functional orientation.

The relationship between lipid domain formation and the electrostatic coupling of cytochrome *c* to lipid monolayers at the air/water interface is the subject of a very recent combined neutron reflectivity/infrared reflection-absorption spectroscopy study [17••]. The monolayer consisted of mixtures of DMPG or dipalmitoylphosphatidylglycerol (DPPG) and DMPC or DPPC. Both chain-protonated and chain-deuterated lipids were used for the neutron reflectivity experiments. The purpose of the study was to determine how protein binding to the monolayer is affected by the composition and phase state of the lipid mixtures. For monolayers containing 30 mol% DMPG, the protein binding was highly dependent on the phase state of the lipid, binding more readily to lipids in the gel phase. This is thought to be due to domain formation in the monolayer due to demixing of the two lipids. This effect had already been demonstrated for the binding of soluble proteins to supported mixed lipid bilayers [18]. The extension of the result to lipid monolayers may lead to yet another

method for the separation of soluble proteins from solution.

4. Peptide / protein interactions with supported single bilayer membranes

Single bilayer model membranes are arguably more biomimetic than lipid monolayers, since they more accurately represent the bilayer nature of cell membranes. Supported single bilayers can be deposited on solid flat substrates from lipids spread on a water surface, using the Langmuir–Blodgett or Langmuir–Schaeffer technique [12], from vesicles in solution [19], or from both, using a combination of these techniques [20]. Peptides or proteins can either be introduced into the solution or incorporated into the vesicles or lipids spread on a water surface in order to study lipid–peptide interactions in these model membrane systems.

An early example of the study of peptide interactions with model membranes is an investigation of streptavidin binding to a biotinylated lipid monolayer spread on top of three layers of cadmium arachidate (3CdA) that had been deposited on a gold-coated silicon substrate [21]. The lipid layer was formed by depositing a mixture of DPPC and biotin-DPPE onto the 3CdA layer using the Langmuir–Blodgett–Kuhn (LBK) technique [22] and was studied in the fluid liquid crystalline phase. The formation of a well-ordered protein monolayer, with the biotin portion of the functionalized lipids embedded in the binding pocket of the protein, was demonstrated with a combination of neutron reflectivity and surface plasmon resonance experiments. Model calculations showed that a more detailed picture of the internal structure of the system could only be obtained with neutron reflectivity, since deuterated lipid molecules could be used.

More recent work in this area [23,24••] has involved judicious use of the contrast variation technique in order to obtain finer structural detail in model bilayer membranes. Neutron and X-ray reflectivity have been used to study the interaction between peptides and model membrane systems consisting of single lipid bilayers of DPPC and mixtures of DPPC with the negatively charged lipid, dipalmitoylphosphatidylserine (DPPS), supported on a silicon substrate. A 16 amino acid peptide, the third helix of the Antennapedia homeodomain, *p-ANTp*$_{43-58}$, was used in these studies. By considering all of the extensive contrast variation data simultaneously in the structure-modeling process, details on the order of 1 Å could be resolved.

In the absence of the peptide, both lipids were found to have the same structure. A thin (5 ± 1 Å) water layer was detected between the silicon substrate and the bilayers. The addition of peptide to the DPPC bilayer produced little change in thickness or roughness of the bilayer. The resultant SLD profiles led to the conclusion that the peptide is most likely segregated in the holes formed due to incomplete coverage of the substrate and does not associate with the lipid. However, the addition of peptide in the mixed DPPC/DPPS bilayer more than doubled the bilayer roughness. In this case, the SLD profiles suggested that the peptide is mainly confined to the lipid headgroups. Thus, the peptide does not associate with the acyl chains in either bilayer.

Other recent work [25••] on peptide/model membrane interactions has used neutron reflectivity to study structural details in hybrid bilayer membranes (HBMs) [26] in D_2O solution in the absence and presence of melittin. The HBMs were formed using a modified Langmuir–Blodgett technique to deposit a layer of chain-deuterated DMPC onto a layer of alkanethiol that had been self-assembled on a gold-coated silicon substrate. The alkanethiol used in this HBM is a novel molecule in which a hydrophilic hexaethyleneoxide moiety is incorporated between the sulfur moiety at the gold surface and the alkane chain [27]. Melittin, a small peptide toxin from bee venom, is often used as a model peptide for antibiotics and membrane proteins. This work is an extension of earlier experiments [28•] on similar HBMs, which represented the first neutron reflectivity experiments on a single lipid bilayer formed with complete surface coverage on a planar substrate.

In order to obtain Angstrom-level information about the structure of these HBMs, improvements in instrumentation and the sample environment were made to allow reflectivity data to be obtained down to 10^{-8} in reflected intensity and out to Q values as high as 0.7 Å^{-1}. The result of these improvements can be seen in Fig. 1, where the reflectivity data from the HBMs in the presence and absence of melittin are shown. The structures of the HBMs were quantified by comparing the resultant SLD profiles to profiles generated by molecular dynamics simulations [29•] of similar HBMs. It was found that the alkanethiol layer exhibits roughness conformal with that of the gold surface. However, this roughness does not propagate throughout the DMPC phospholipid layer. Rather, the more-fluid DMPC layer essentially anneals, smoothing out the roughness present in the alkane region.

The SLD profiles obtained for the HBMs from the reflectivity data using a model-independent fitting method [5] are shown in Fig. 2. They show unequivocally that the ethylene oxide region of the alkanethiol layer is not hydrated with water from the solution. Furthermore, it is evident that melittin does not alter

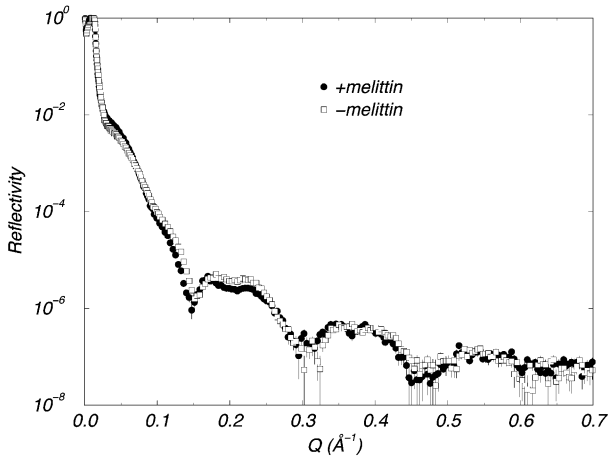

Fig. 1. Reflectivity from thiahexaethyleneoxide-alkane/DMPC HBMs. Measured neutron reflectivity vs. Q from thiahexaethyleneoxide-alkane/DMPC HBMs in the presence (●) and absence (□) of the membrane-active peptide, melittin, in contact with D_2O solution. Chain-deuterated DMPC molecules were used to increase the contrast between the alkanethiol and phospholipid layers.

the HBM in a way that allows bulk water to penetrate into this region. The location of melittin appears to be primarily in the DMPC headgroups. The large change in SLD suggests that there is significantly less water in this region when melittin is present. However, melittin does have a significant effect on the alkanethiol layer since it is found to be approximately 3 Å thicker in the presence of melittin. This can occur if the length of the alkane chains has increased, presumably through a decrease in tilt angle. In spite of the fact that SLD profiles have been determined with unprecedented confidence and detail, the simultaneous presence of water and melittin in the DMPC acyl chain region could not be ruled out in this work. Thus, contrast variation experiments are still needed to determine the amount of melittin and water in all of the layers.

5. Phase-sensitive neutron reflectometry for the measurement of biomimetic membranes

Recently, an exciting new phase-sensitive neutron reflectivity method that allows the determination of the compositional depth profiles of biomimetic membranes from first principles, without the need for fitting, has been reported [30••]. Two reflectivity data sets were collected for identical alkanethiol/DMPC HBMs that were formed on gold-coated silicon and sapphire substrates. The neutrons are incident through the highly-transparent silicon or sapphire substrate, which becomes the 'fronting' medium in these experiments. By so varying the composition, and thus the SLD, of the fronting medium, it is possible to obtain the complex reflection amplitude, which includes the phase information, for the HBM. Determination of the complex reflection amplitude using this surround variation method [31] allows the related SLD profiles to be obtained by first-principles inversion without the need for fitting or adjustable parameters. The SLD profile obtained in this way is ensured to be unique and it compared favorably with that calculated from the chemical compositional profile predicted by a molecular dynamics simulation of an HBM of similar structure [29•]. Again, it should be emphasized that this new experimental method, which yields exact SLD profiles, must still be combined with the contrast variation technique to obtain information on the location of protein and water in all of the regions of the HBM.

6. Concluding remarks

The neutron reflectivity technique has been shown to be an essential tool for the characterization of biological and biomimetic thin films. Thanks to improvements in the experimental technique over the last few years, it is now possible to determine accurate compositional depth profiles with Angstrom-level detail. The phase-sensitive measurement technique, combined with contrast variation studies, promises an unprecedented level of accuracy and detail in the study of peptide and protein interaction with model

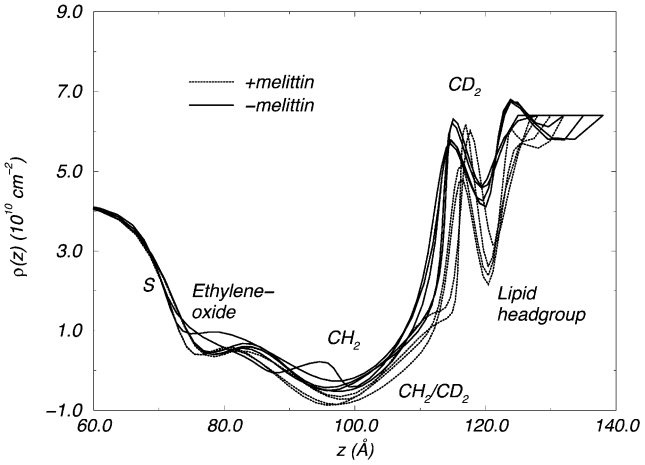

Fig. 2. Neutron SLD profiles from thiahexaethyleneoxide-alkane/DMPC HBMs. 'Families' of neutron SLD profiles, $\rho(z)$ vs. z, for thiahexaethyleneoxide-alkane/DMPC HBMs in the presence (---) and absence (—) of melittin, obtained from the reflectivity data in Fig. 1. In each case, the family of SLD profiles was obtained from multiple fits to the data using a model-independent fitting method [5]. Each SLD profile represents an equally good fit to the corresponding reflectivity data. The silicon substrate is located at $z = 0$ Å, by definition. The region representing the gold coating (0 Å $\leq z \leq$ 60 Å) is omitted in the SLD profiles so that the changes in the HBM region can be observed more clearly.

biological membranes. Such experiments are now feasible and are limited only by the ability to prepare suitable biomimetic samples with embedded proteins or peptides.

References and recommended reading

- • of special interest
- •• of outstanding interest

[1] Majkrzak CF, Felcher GP. Neutron scattering studies of surfaces and interfaces. Mater Res Soc Bull 1990;15:65–72.

[2] Penfold J, Thomas RK. The application of the specular reflection of neutrons to the study of surfaces and interfaces. J Phys: Condens Matter 1990;2:1369–1412.

[3] Majkrzak CF. Fundamentals of specular neutron reflectometry. Mat Res Soc Symp Proc 1995;376:143–156.

[4] Penfold J, Richardson RM, Zarbakhsh A et al. Recent advances in the study of chemical surfaces and interfaces by specular neutron reflection. J Chem Soc Faraday Trans 1997;93:3899–3917.

[5] Berk NF, Majkrzak CF. Using parametric B splines to fit specular reflectivities. Phys Rev B 1995;51:11296–11309.

[6] • Bayerl TM, Thomas RK, Penfold J, Rennie A, Sachmann E. Specular reflection of neutrons at phospholipid monolayers. Biophys J 1990;57:1095–1098.

First neutron reflectivity measurements of lipid monolayers at the air/water interface. This work shows the importance of neutrons in pinpointing the location of water in the lipid headgroups.

[7] Vaknin D, Kjaer K, Als-Nielsen J, Lösche M. Structural properties of phosphatidylcholine in a monolayer at the air/water interface: neutron reflection study and reexamination of X-ray reflection measurements. Biophys J 1991; 59:1325–1332.

[8] Koenig BW, Krueger S, Orts WJ et al. Neutron reflectivity and atomic force microscopy studies of a lipid bilayer in water adsorbed to the surface of a silicon single crystal. Langmuir 1996;12:1343–1350.

[9] • Johnson SJ, Bayerl TM, McDermott DC et al. Structure of an adsorbed dimyristoylphosphatidylcholine bilayer measured with specular reflection of neutrons. Biophys J 1991;59:289–294.

First neutron reflectivity experiments of a supported single lipid bilayer in aqueous solution. Such in situ measurements are possible because neutrons interact weakly with materials and the solid support can be used as the incident medium, allowing the bilayer to be in full contact with water.

[10] • Krueger S, Ankner JF, Satija SK, Majkrzak CF, Gurley D, Colombini M. Extending the angular range of neutron reflectivity measurements from planar lipid bilayers: application to a model biological membrane. Langmuir 1995;11: 3218–3222.

First neutron reflectivity experiment on a supported single lipid bilayer to use a novel silicon wafer and water 'sandwich' to decrease the volume of the water reservoir in contact with the sample. This greatly decreased the background from incoherent scattering from hydrogen and deuterium, allowing measurements to be made out to more than twice the Q value of previous experiments.

[11] •• Charitat T, Bellet-Amalric E, Fragneto G, Graner F. Adsorbed and free lipid bilayers at the solid-liquid interface. Eur Phys J B 1999;8:583–593.

First study showing the formation of robust, reproducible double bilayers, in which the second bilayer is floating freely on top of the first. Since the second bilayer is free-floating, this experimental system promises to be a more biomimetic model membrane than supported single bilayers since it has the potential to support transmembrane proteins. It is not clear from this initial experiment whether this system will maintain its integrity in the presence of proteins or peptides. However, the results of further ongoing work should answer this question.

[12] Tamm LK, McConnell HM. Supported phospholipid bilayers. Biophys J 1985;47:105–113.

[13] Vaknin D, Als-Nielsen J, Piepenstock M, Lösche M. Recognition processes at a functionalized lipid surface observed with molecular resolution. Biophys J 1991;60:1545–1552.

[14] Vaknin D, Kjaer K, Ringsdorf H et al. X-ray and neutron reflectivity studies of a protein monolayer adsorbed to a functionalized aqueous surface. Langmuir 1993;9:1171–1174.

[15] Lösche M, Piepenstock M, Diederich A, Grünewald T, Kjaer K, Vaknin D. Influence of surface chemistry on the structural organization of monomolecular protein layers adsorbed to functionalized aqueous interfaces. Biophys J 1993;65: 2160–2177.

[16] •• Naumann C, Dietrich C, Behrisch A et al. Hisactophilin-mediated binding of actin to lipid lamellae: a neutron reflectivity study of protein membrane coupling. Biophys J 1996;71:811–823.

This work showed neutron reflectivity could be used to definitively show that native hisactophilin partially penetrated into the monolayer, such that the fatty acid groups on the protein bound tightly to the lipid. On the other hand, when these groups were absent in the hisactophilin mutant, the protein interacted with the lipid through electrostatic interactions, forming an adsorbed layer on top of the lipid monolayer. Taking into account the known structure of hisactophilin, it could be deduced that the mutant protein was bound in a different orientation than the native protein.

[17] •• Maierhofer AP, Bucknall DG, Bayerl TM. Modulation of cytochrome *C* coupling to anionic lipid monolayers by a change of phase state: a combined neutron and infrared reflection study. Biophys J 2000;79:1428–1437.

A good example of the complementary use of neutron reflectivity and reflection-absorption infrared spectroscopy. The monolayer structure and the amount of cytochrome c bound to the monolayers was obtained directly from the reflectivity measurements, while the kinetics of protein adsorption was studied by infrared spectroscopy.

[18] Käsbauer M, Bayerl TM. Formation of domains of cationic or anionic lipids in binary/lipid mixtures increases the coupling strength of water soluble proteins to supported bilayers. Biochemistry 1999;38:15258–15263.

[19] Brian AA, McConnell HM. Allogeneic stimulation of cytotoxic T cells by supported planar membranes. Proc Natl Acad Sci USA 1984;81:6159–6163.

[20] Kalb E, Fray S, Tamm LK. Formation of supported planar bilayers by fusion of vesicles to supported phospholipid bilayers. Biochim Biophys Acta 1992;1103:307–316.

[21] Schmidt A, Spinke J, Bayerl T, Sachmann E, Knoll W. Streptavidin binding to biotinylated lipid layers on solid supports. Biophys J 1992;63:1185–1192.

[22] Kuhn H, Möbius D, Bücher H. Spectroscopy of monolayer assemblies. In: Weissberger A, Rossiter BW, editors. Physical methods of chemistry. New York: Wiley, 1972.

[23] Fragneto G, Bellet-Amalric E, Charitat T, Dubos P, Graner F, Perino-Galice L. Neutron and X-ray reflectivity studies at solid–liquid interfaces: the interaction of a peptide with model membranes. Physica B 2000;276-278:501–502.

[24] •• Fragneto G, Graner F, Charitat T, Dubos P, Bellet-Amalric E. Interaction of the third helix of Antennapedia homeodomain with a deposited phospholipid bilayer: a neutron reflectivity structural study. Langmuir 2000;16:4581–4588.

A careful study of interaction of a small peptide with supported single lipid bilayers. Extensive contrast variation measurements were performed to obtain a model structure that fit all of the

contrast variation data simultaneously. Although these experiments were performed with the lipids in the gel phase, current work is focussed on the physiologically-relevant liquid crystalline phase.

[25] •• Krueger S, Meuse CW, Majkrzak CF et al. Investigation of hybrid bilayer membranes with neutron reflectometry: probing the interactions of melittin. Langmuir 2000;17:511–521.

The first neutron reflectivity measurements on single hybrid bilayer membranes (HBMs) in which reflectivities down to 10^{-8} and out to $Q = 0.7$ Å^{-1} were measured. Under these conditions, the resolution of the neutron reflectivity experiments is now limited only by the roughness of the supporting substrate. Extensive model fitting allowed the SLD profiles for the HBMs in the absence and presence of a membrane-active peptide to be obtained with unprecedented accuracy and detail. Since care was taken to obtain a 'family' of fitted SLD profiles for each HBM system, the variations in the sensitivity of the neutron reflectivity technique from region-to-region could easily be tracked

[26] Plant AL. Self-assembled phospholipid alkanethiol biomimetic bilayers on gold. Langmuir 1993;9:2764–2767.

[27] Vanderah DJ, Meuse CW, Silin V, Plant AL. Synthesis and characterization of self-assembled monolayers of alkylated 1-thiahexa(ethylene oxide) compounds on gold. Langmuir 1998;14:6916–6923.

[28] • Meuse CW, Krueger S, Majkrzak CF et al. Hybrid bilayer membranes in air and water: infrared spectroscopy and neutron reflectivity studies. Biophys J 1998;74:1388–1398.

The first neutron reflectivity experiments on a hybrid bilayer membrane (HBM). The interpretation of the resultant SLD profiles was simplified since the HBM is formed on the silicon substrate with complete surface coverage and since complementary reflection-absorption infrared spectroscopy measurements allowed an independent determination of the tilt angle of the alkane chains.

[29] • Tarek M, Tu K, Klein ML, Tobias DJ. Molecular dynamics simulations of supported phospholipid/alkanethiol bilayers on a gold (111) surface. Biophys J 1999;77:964–972.

First molecular dynamics simulation of a supported, tethered biomimetic membrane.

[30] •• Majkrzak CF, Berk NF, Krueger S et al. First-principles determination of hybrid bilayer membrane structure by phase-sensitive neutron reflectometry. Biophys J 2000; 79:3330–3340.

Detailed explanation of phase-sensitive neutron reflectometry experimental methods with a demonstration of how the 'surround variation' method works using a hybrid bilayer membrane sample. The paper also shows how the real part of the complex reflection amplitude can be used to assign a spatial resolution to the SLD profile for a given Q-range over which the data are collected. The use of the imaginary part of the complex reflection amplitude as a sensitive diagnostic for recognizing the existence of certain in-plane inhomogeneities in the sample is also demonstrated.

[31] Majkrzak CF, Berk NF, Silin V, Meuse CW. Experimental demonstration of phase determination in neutron reflectometry by variation of the surrounding media. Physica B 2000;283:248–252.