



## Neutron reflection from supported lipid membranes

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### ABSTRACT

Neutron reflection has become a popular tool to study supported lipid membranes, demonstrating the advantages of the structural and compositional insights given by H/D contrast variation in biophysical membrane studies. While technical advances such as magnetic contrast variation and new data-analysis techniques have increased the accuracy of data modeling and interpretation, the use of complementary techniques has widened the range of membrane applications studied and allowed the investigation of more complex systems. This review describes the major technical developments in the membrane systems studied as well as their rapidly increasing number of applications.

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### 1. Introduction

Biological lipid membranes are perhaps the most abundant self-assembling molecular system in nature – they surround every living cell and form some of the cell machinery, but they also form much larger assemblies in the lining of the lungs, and the complex structure of the skin. Although the fundamental two-dimensional fluid nature of lipid membranes is thought to be most important in their biological function, two equally notable features of cell membranes are their complexity and diversity – each membrane has a unique composition that reflects its biological origin. Due to the vast complexity of cell membranes, model lipid membranes with well-defined composition were developed for studying the structure and interactions of biologically and pharmaceutically relevant membrane components. Supported membranes [1] in particular are used widely because of their ease of formation and manipulation in technological applications.

The emergence of a variety of modern techniques to probe membrane structure and function means that we have access to a range of membrane properties, including lateral structure, mechanical properties, lipid mixing and dynamics. However, despite the number of techniques available, few are able to measure the lipid composition in a membrane, and have limited resolution to proteins relative to the lipids. The main advantage of neutron reflection over other state-of-the-art techniques is the ability to detect the absolute membrane composition and relative location of lipids and proteins in multi-

component membranes. In addition, the technique is emerging as a powerful tool to investigate the orientation and conformation of membrane-bound proteins in a non-crystalline aqueous environment which is particularly valuable in cases where no crystal structures can be obtained.

Neutron reflection probes membrane structure on the Ångström-nanometre scale at which most of the functional interactions between lipids and proteins take place, and is, due to the sensitivity of neutrons to the scattering length density difference of hydrogen and deuterium, an ideal tool to elucidate the composition of multi-component membranes via selective deuteration. In contrast to synchrotron X-ray beams or high intensity lasers, the low-energy neutrons used give rise to no physical damage to the sample and easy access to buried aqueous interfaces. The scattering potential of neutrons only depends on the nuclear composition and thus neutrons are applicable to materials that exhibit optical anisotropy, such as membranes below the gel-to-fluid transition. The neutron wavelengths employed (1–30 Å) are well matched to the length scale of biological membranes and proteins, and reflection allows structures of up to ~1000 Å thick to be investigated. At the time of previous review on the topic [2], the use of neutron reflection in membrane studies was still very much in its infancy, but in the last decade the field has grown significantly, and today neutron reflection is used for studying increasingly complex membrane systems. This review covers the progress made recently in the measurement and analysis techniques applicable to membranes as well as the wide range of new model membranes developed, and their experimental applications.

Specular neutron reflection (with the angle of reflection being the same as the angle of incidence) measures the thickness and scattering length density (or neutron refractive index) profile of a film along the

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direction normal to its surface ( $z$ ). The scattering length density  $\rho(z)$  of a material is the sum of the coherent nuclear scattering lengths  $b_i$  of its constituent atoms and their number density  $n_i(z)$ :

$$\rho(z) = \sum_i b_i n_i(z).$$

Neutron reflectivity data are measured as the reflected intensity relative to the incident beam ( $R = I/I_0$ ) and recorded as a function of the momentum transfer vector  $q_z$ :

$$q_z = \frac{4\pi \sin\theta}{\lambda}$$

where  $\theta$  is the angle of incidence and  $\lambda$  the neutron wavelength. Thus, reflectivity can be measured in two ways: either by using a single wavelength (monochromatic mode) and varying the angle of incidence, or by using a polychromatic beam (time-of-flight mode) and recording reflectivity at a few fixed angles of incidence to cover the desired  $q$  range. The resolution of the experiment is determined by the wavelength resolution as well as the angular resolution of the incident beam, with resolution values of  $dq/q = 0.01$ – $0.05$  typically employed.

In addition to the specularly reflected signal, it is possible that a laterally structured sample gives rise to off-specular reflection, i.e. in addition to momentum transfer in the surface normal direction, there are components parallel to the surface plane. When the off-specular reflections arise from structures that are much smaller than the coherence length of the neutrons ( $\sim 10$ – $100 \mu\text{m}$ ), an average of the lateral scattering length density profile is observed by the specular reflectivity. If the lateral structure has a characteristic length scale much larger than the neutron coherence length, the reflections arising from it are no longer coherent, and have to be treated as the sum of reflections from the independent structures. In the intermediate size range, structures with characteristic dimensions approaching the neutron coherence length lead to significant off-specular scattering, which decreases the specular reflection signal. Off-specular reflection could in principle be used to detect membrane domains, but in reality the membrane domains can be so small (50–100 nm) that the signal (which is also very weak) is not observed in the same detector position as the specular reflection, and manifests itself only as an “interfacial roughness” which slightly decreases the specular intensity. Larger domains lead to observable off-specular reflectivity, but the analysis of off-specular reflectivity in general is still in its infancy. Time-of-flight grazing incidence small angle neutron scattering (GISANS) [3] using a laterally well collimated beam ( $\sim \text{mm}$ ) has been successfully applied to determine the lateral structure of polymer films, but these are typically much thicker than a lipid bilayer and similar experiments on membranes have yet to be realized. It is also possible to encode the off-specular scattering signal by employing neutron spin interaction with a magnetic field and the emerging technique of spin echo grazing incidence scattering (SERGIS) has likewise been applied to polymer films [4].

In neutron reflectivity from supported membrane systems, the solid material is chosen to be highly transparent to neutrons so that the membrane–water interface can be approached through the solid to avoid scattering in the bulk liquid phase. The reflectivity contribution from the solid material (most commonly silicon, quartz or sapphire) can be eliminated by solvent contrast matching, but as reflectivity is most sensitive to differences in scattering length density, this is often used as a means to boost the signal from the sample. Interfacial roughness is the main limiting factor in reflectivity studies, and the solid substrates need to be polished to a high degree of smoothness ( $\text{rms} < 5 \text{ \AA}$ ) and flatness.

In a supported membrane, the scattering length density profile (sld) normal to the interface  $\rho(z)$  is modeled in terms of the contributions from the solid surface, the lipid chains, headgroups

and the hydration present, which depends on the thickness and packing density of the lipids. In addition, proteins or other additive components may contribute to the sld. In a multi-component layer, the scattering length density is described in terms of the volume fraction  $\phi$  and molecular sld  $\rho_i$  of the components, for example in a lipid–protein layer the total sld would be:

$$\rho_{\text{layer}} = \phi_{\text{lipid}}\rho_{\text{lipid}} + \phi_{\text{protein}}\rho_{\text{protein}} + \phi_{\text{water}}\rho_{\text{water}}$$

where  $\phi_{\text{lipid}} + \phi_{\text{protein}} + \phi_{\text{water}} = 1$ . The molecular scattering length densities are calculated from the sum of nuclear scattering lengths and the molecular volumes. For lipids this can be done in terms of the component volumes of the headgroups and the chains, or smaller fragments, which can be obtained for example from molecular dynamics simulations of lipid bilayers [5] or from X-ray diffraction data of lyotropic lipid phases in the bulk. The volume fractions of individual components can be found by recording reflectivity profiles in several solvent contrasts, provided that the molecular scattering length densities are sufficiently different from one another (the resolution in scattering length density is typically  $\pm 0.2 \times 10^{-6} \text{ \AA}^{-2}$  if data are recorded in multiple contrasts). In more complex membranes, e.g. on a polymer cushion, the structure of the cushion is typically determined before membrane formation, and the polymer cushion is usually distinguishable from the lipid membrane due to high solvent content, or selective deuteration. Smaller components of the membrane, such as counterions are usually not detected, as they are present in low densities.

## 2. Experimental and analysis methods

### 2.1. Experimental methods

Neutron reflectivity data from membranes are equally well obtained on monochromatic and time-of-flight reflectometers, with the limiting factor being the accessible  $q$  range and background of the instrument, which influence the choice of instrument and/or membrane system. To resolve the thickness and composition of a membrane, access to a measurable reflectivity signal over a  $q$  range covering at least the first minimum in the neutron reflectivity profile is necessary, and this is one of the key challenges in using single lipid membranes in contact with bulk water. On the other hand, it is also the reason why solid-supported membranes are advantageous in neutron reflectivity, because the first minimum is usually observable even for single lipid bilayers, whereas for lipid monolayers at the free liquid interface it is practically out of reach of neutron reflectivity.

The incoherent scattering by the bulk liquid subphase is the main source of background in neutron reflectivity, although instrumental contributions (air scattering, windows etc.) to the background also exist. Reduction of the incoherent background is possible by employing ultra-thin solvent reservoirs [6], which allow reflectivities down to  $10^{-8}$  to be recorded and increases the accessible  $q$  range [7]. In addition, the nature of the solid surface may be tuned to give additional contrast or sensitivity to the membrane structure, for example with a highly reflective gold coating of a thickness that shifts the reflectivity oscillations to a lower  $q$  range where the reflected intensity is higher and small thickness changes are more easily observable. However, there is a clear trend towards time-of-flight (TOF) reflectivity in membrane studies because the primary interest often lies in following changes in membrane structures, and thus the majority of new instruments for soft matter and biological applications operate in TOF mode.

Solvent contrast variation is most commonly employed to increase the resolution of neutron reflectivity to supported membrane structure, but with the increasing use of multi-component membranes, and the emergence of several deuteration facilities around the

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