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Studies of model biological and bio-mimetic membrane structure: Reflectivity vs diffraction, a critical comparison

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ABSTRACT

Recent years have seen very significant progress made in the application of X-ray and neutron diffraction and reflectivity in structural studies of lipid and lipid–protein membranes. Improvements in instrumentation and the development of new sample preparation techniques and specialized sample environments have afforded data that provide a greater resolution of structural detail, and in many cases on systems that have a complexity of composition and architecture that closely mimic those of true biological membranes. This review provides an overview of the various methodologies involved in membrane reflectivity and diffraction experiments, with a primary focus on aspects of sample preparation and data analysis. We then provide a review of some of the research performed in this area over the period 2010–2015, offering a critical comparison of reflectivity vs. diffraction experiments.

1. Introduction

Structural studies of the complex protein–lipid matrices that comprise biological membranes are key not only to our understanding of living systems—how they work, and how they malfunction in disease—but also in our quest to develop self-assembling bio-mimetic membrane systems that can be exploited to aid research in drug delivery and in the development of clinical diagnostics or as components of biosensors. The atomic structures of the individual components of these biological and bio-mimetic membranes, components such as sterols, glycolipids and lipoproteins, for example, can be successfully determined using X-ray crystallography or high field nuclear magnetic resonance spectroscopy. The same high resolution techniques, however, cannot be brought to bear to provide the same level of structural detail on a complete membrane assembly: the assemblies are essentially fluid-like and lack long range order. To study the structures of membrane ensembles, therefore, researchers must use more appropriate techniques, and arguably the most useful of these are those of X-ray and neutron reflectivity and diffraction.

In this report, we first present an overview of the various methodologies involved in membrane reflectivity and diffraction experiments, with a primary focus on aspects of sample preparation and data analysis. We then provide a review of some of the research performed in this area over the period 2010–2015, offering a critical comparison of reflectivity vs. diffraction experiments. In view of the volume of research published in this area, we confine ourselves to a review of research on model *biological* and *bio-mimetic* membrane *bilayer* systems only, and we do not discuss any of the studies reported wherein Langmuir monolayers (formed at an air–water interface) have been used as surrogates for biological membranes, nor any of the studies that deal with membranes made from synthetic surfactants or synthetic or natural polymers.

The interested reader might also refer to the earlier reviews by Harroun et al. [1], Penfold [2], and Wacklin [3].

2. X-ray and neutron diffraction studies

2.1. Experimental aspects

The diffraction of X-rays or neutrons from a stack of aligned membrane bilayers (see below) is governed by Bragg's law:

$$h\lambda = 2d\ \sin\left(\frac{\theta}{2}\right) \tag{1}$$

where d (the so-called d-spacing) is the distance of separation between successive lattice planes (that is, the distance between successive bilayers), h is the diffraction order, λ is the wavelength of the incoming X-rays or neutrons, and $\theta/2$ (the Bragg angle) is the angle between the lattice plane and the diffracted beam (Fig. 1).

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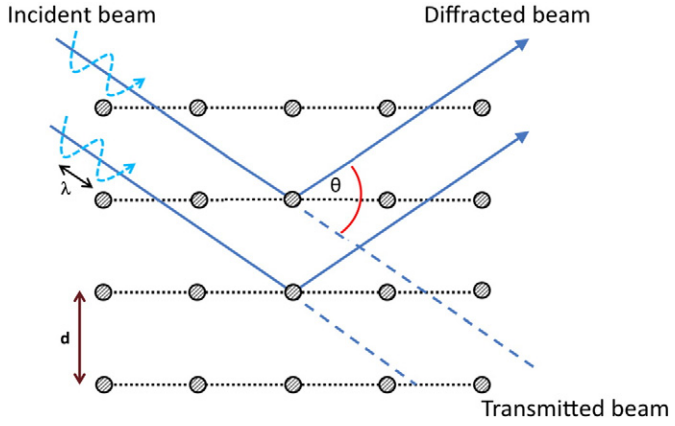

Fig. 1. Schematic representation of the diffraction phenomenon. The lattice planes are separated by a distance *d*, and the incident X-rays or neutrons of wavelength, λ, are diffracted through an angle, *θ/2*.

For the case of neutron diffraction, the corrected intensities of the various Bragg reflections/diffraction peaks ($I_{cor}(h)$) are obtained from the observed intensities ($I_{obs}(h)$) as:

$$\mathbf{I}_{cor}(\mathbf{h}) = \frac{\mathrm{I}_{obs}(\mathbf{h}) \sin \mathbf{2}\theta}{A_h(h)B(h)} \tag{2}$$

where $I(h)$ is the total intensity of the diffraction peak, $B(h)$ is the acceptance correction that is a function of the detector aperture dimensions—and generally taken to be unity—while $A_h(h)$ is the correction factor for sample adsorption [4]:

$$\mathbf{A}_h(\mathbf{h}) = \frac{\sin\theta}{2\mu T} \cdot \left(\mathbf{1} - e^{\frac{-2\mu T}{\sin\theta}}\right) \tag{3}$$

$A_h(h)$ is dependent upon the diffraction angle (θ), and the sample thickness (T) and its linear attenuation coefficient (μ—which is calculated from the neutron wavelength, in combination with the sample composition and density) [4].

The amplitudes of the structure factors for the diffracting sample, $F_h(h)$ are obtained from the corrected Bragg peak intensities, I_{cor} as [5]:

$$|\mathbf{F}_h(\mathbf{h})| = \sqrt{I_{cor}(h)h}. \tag{4}$$

The Lorentz factor, $L(h)$, is computed as $sin2\theta$; but is conveniently approximated as h at small angles [5].

The neutron scattering length density profile ($\varphi(z)$) in the direction normal to the sample surface (that is, normal to the membrane plane) (z) is given by the Fourier summation:

$$\varphi(\mathrm{z}) = \frac{2}{d}\sum_{h=1}^{h_{max}} |F_h(h)|\epsilon(h) \cos\left(2\pi\frac{hz}{d}\right) \tag{5}$$

where $\varepsilon(h)$ are the phases of the structure factors.

For the case of X-ray diffraction, the same equations apply but $\varphi(\mathrm{z})$ then yields the profile of *electron* density across the lamellar unit cell.

The scattering density ($\varphi(z)$) obtained *via* Eq. (5) is given on a relative scale with respect to the unit cell components and takes into account only the fluctuation of φ across the cell. In order to put $\varphi(z)$ on an absolute scale, one must either compute the difference profile for matching protiated and selectively deuterated samples and make appropriate adjustments arranging that the integrated differences give the known scattering length density of the deuterated moiety, or else translate the profile so that the mean density, $\phi(0)$, is shifted as the average scattering density per unit length of bilayer (computed from the sample composition).

2.2. Solving the phase problem

As noted above, the use of aligned multilayer diffraction data to derive the scattering density along the direction normal to the membrane plane (z) requires a Fourier summation (Eq. (5)) involving the structure factor amplitudes, $|F_h|$, and the corresponding phase angles, $\varepsilon(h)$. The values of $|F_h|$ are measurable—derived from the intensities of the Bragg reflections—but the corresponding $\varepsilon(h)$ are not, and in the general case can take any value in the range 0 to 2π. Where the system studied is centrosymmetric, however, such that $\phi(z) = -\phi(-z)$—which is fortunately the case for aligned membrane stacks—the phase angles are restricted as 0 or π and so the signs of the phases (by trigonometric identity) can only take values of $+1$ or -1 [6]. For a diffraction pattern with n measured Bragg reflections this gives 2^n combinations of phase signs.

In neutron diffraction studies, the correct choice of phase signs is determined simply by recording the diffraction pattern with samples hydrated with H_2O, D_2O and/or H_2O:D_2O mixtures. Assuming that the water in the system (or at least the majority of it) is localized at a centre of symmetry (trapped between successive bilayers), the increase in density afforded by the addition of deuterium (as D_2O) leads to a positive value added to each of the structure factors. The structure factors for which $\varepsilon(h)$ is positive, therefore, will increase in magnitude, whereas negative structure factors will show a decrease. Hence, simply by noting whether Bragg reflections increase or decrease in intensity when a sample is switched from H_2O to D_2O will allow $\varepsilon(h)$ to be determined. For the weaker reflections, however, there may be difficulties that arise because the D_2O addition may cause a change in the sign of $\varepsilon(h)$. This ambiguity is resolved by measuring the diffraction pattern with at least three different solvent H/D contrasts—researchers generally opting for H_2O, D_2O and 50:50 H_2O:D_2O. For centrosymmetric structures, a plot of the signed structure factor amplitudes against $\%D_2O$ will then be linear [7] and any change in phase sign can be readily identified (see Fig. 2a).

Confirmation of phase signs can also be made using the theoretical calculations presented by Léonard et al., which allow that the slope of the plot of $|F_h|$ vs. $\%D_2O$ can be predicted as positive or negative given an estimate of d_w/d, where d is the bilayer d-spacing and d_w, the expected inter-bilayer water layer thickness [8].

In the case of X-ray diffraction studies of membranes, the device of changing the H/D solvent contrast can not be used to solve the phase problem because the scattering of X-rays by H and D is much the same. Use is made instead of the so-called swelling method wherein a given sample is equilibrated at different relative humidities (RH) achieved using a hydration chamber containing a reservoir of various salt solutions. The resulting small changes in hydration are then assumed to cause small changes in the inter-bilayer water layer thickness but leaving the bilayer structure itself largely unaffected. Such an assumption is not exactly true but is generally accepted as a good first approximation. It then follows from Shannon's sampling theorem [9] that if the structure factor amplitudes of the Bragg reflections recorded under each RH, $|F_{h,RH}|$, are plotted against the positions of the reflections., $q_{h,RH}$, this discrete set of data points should sample the continuous transform, $F(q)$:

$$\mathbf{F}(\mathbf{q}) = \sum \varphi_h |F_{h,\,RH}| \sin\frac{\frac{qd}{2} - \pi h}{\frac{qd}{2} - \pi h} \tag{6}$$

re-constructed with appropriately phased $F_{h,RH}$ at a given RH, with $F_{0,RH}$ set as the positive average electron density of the sample lamellae (see Fig. 2b).