Phase-sensitive specular neutron reflectometry for imaging the nanometer scale composition depth profile of thin-film materials

B.J. Kirby a, P.A. Kienzle a, B.B. Maranville a, N.F. Berk a, J. Krycka a, F. Heinrich b, C.F. Majkrzak a,*

a NIST Center for Neutron Research, Gaithersburg, MD 20899, United States
b Physics Department, Carnegie Mellon University, Pittsburgh, PA 15213, United States

ARTICLE INFO

Article history:
Received 29 August 2011
Accepted 13 November 2011
Available online 20 November 2011

Keywords:
Neutron Reflectivity
Phase-sensitive Thin films Multilayers Composition depth profile

ABSTRACT

Neutron reflectometry is a powerful method for probing the molecular scale structure of both hard and soft condensed matter films. Moreover, the phase-sensitive methods which have been developed make it possible for specular neutron reflectometry to be effectively employed as an imaging device of the composition depth profile of thin film materials with a spatial resolution approaching a fraction of a nanometer. The image of the cross-sectional distribution of matter in the film obtained in such a way can be shown to be, in most cases, unambiguous to a degree limited primarily by the range and statistical uncertainty of the reflectivity data available. The application of phase-sensitive neutron reflectometry (PSNR) to the study of several types of soft matter thin film systems are illustrated by a number of specific examples from recent studies. In addition, new software tools available to the researcher to apply PSNR methods and analysis are discussed.

1. Introduction

Over the past quarter century or so, neutron reflectometry (NR) has become an established probe of the nanometer scale structure of materials in thin film and multi-layered form. NR has contributed to our understanding of layered systems of soft condensed matter of interest in polymer science, organic chemistry, and biology and of magnetic and superconducting hard condensed matter film systems [1–15]. In general, neutrons are especially sensitive to hydrogen and atomic magnetic moments, thereby potentially complementing sub-surface structural information that can be obtained from X-ray reflectivity measurements. In particular for NR, the scattering length density (SLD) depth profile along the surface normal, averaged over in plane, can be deduced from specular neutron reflectivity measurements (wavevector transfer Q normal to the surface). The SLD is the sum of the individual products of the number of atoms of each isotope per unit volume and its corresponding neutron scattering strength as characterized by a scalar number called the coherent scattering length. This number may be complex if absorption occurs.) Under nearly ideal conditions, neutron reflectivities as low as $10^{-8}$ out to a Q of 0.7 Å$^{-1}$ from a lipid bilayer membrane have been measured with a corresponding spatial resolution in the SLD profile of half a nanometer [16]. The SLD profile is directly related to the corresponding material composition distribution and if polarized neutron beams are employed, the vectorial magnetization depth profile of magnetic materials can be obtained as well.

Moreover, it has been shown relatively recently that in specular neutron reflectometry the phase can be determined exactly using reference structures, thereby enabling a first-principles inversion, and thus ensuring a unique result for the SLD profile [17–23]. (Other schemes for retrieving phase information in reflectivity measurements have also been developed [24–26], but these do not enable the direct inversion described here.) The ability to establish an unambiguous correspondence between reflectivity data and a SLD profile is immensely powerful and one of the most important factors in making an accurate structure determination. The experimentally determined reflectivity is the reflected intensity divided by that of the incident beam. Because the reflectivity is the square of the modulus of the reflected wave, the phase of the reflected wave cannot explicitly express any information about the phase of the reflected wave. The phase of the reflected wave contains essential structural information without which a unique solution for a given structure cannot be determined in all cases. Some examples of non-unique SLD profiles associated with single reflectivity data sets are presented in [27–29]. The phase-sensitive methods which have been developed allow specular neutron reflectometry to be effectively employed as an imaging device of the composition depth profile of thin film materials with a spatial resolution approaching a fraction of a nanometer. The image of the cross-sectional distribution of matter in the film obtained in such a way can be shown to be, in the vast majority of cases, unambiguous to a degree limited primarily by the range and statistical uncertainty of the reflectivity data available [30]. The uniqueness follows from the mathematical one-to-one correspondence which exists between the reflection amplitude and the scattering length density (SLD) distribution of the film structure along the surface normal. This is an extraordinary relationship

* Corresponding author.

1359-0294/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.cocis.2011.11.001
for what is fundamentally a diffraction process and one which is enabled by the use of external reference media placed adjacent but external to the film of interest—the SLD depth profile of which is initially unknown. The reference media may be a variable “fronting” or “backing” medium on either side of the film of interest (one of which can also serve as supporting substrate) or a separate, variable reference film of finite thickness (such as a saturated ferromagnetic film). This one-dimensional holographic (interferometric) technique can be applied to film materials of both hard and soft condensed matter and is especially sensitive to hydrodynamic organic and magnetic systems. In cases involving organic films where deuterium substitution can be performed isomorphically, the phase-sensitive methods applied to both protonated and deuterated versions of the sample can enhance the accuracy of the structural information deduced from the reflectivity measurements. For example, if two segments of the depth profile of an organic film have different chemical compositions but happen to have nearly the same SLD, then deuterating one segment and protonating the other isomorphically would in principle enable NR to distinguish one from the other. Alternatively, the isomorphic nature of such an exchange can be validated in a structure that is already known a priori. General discussions of PSNR methods can be found, for example, in [31,32].

One of the principal aims of this article is to enable the researcher to access the tools now available to apply these phase-sensitive neutron reflectometry (PSNR) methods to current materials research problems involving thin film systems. Although numerous “proof-of-principle” demonstration experiments and analyses have been performed [33–37], application of phase-sensitive methods to scientific problems on the forefront of current research has not yet been widespread. Possible reasons for this include the relatively formidable mathematics of the inversion procedure and stringent requirements on the quality of the sample (particularly regarding in-plane homogeneity). The former potential obstacle has been largely removed with newly available software packages for performing such calculations and by the introduction of an alternative means of analysis which employs simultaneous fitting of multiple data sets. The latter impediment can be eliminated to a certain extent by high-quality, well-characterized standard reference substrates. Efforts to develop, and make available for general use, such substrates are currently underway at the NIST Center for Neutron Research (NCNR), e.g., magnetic Permalloy and Au layers on Si. No matter how accurately the NR measurements are performed, the quality of the result can be no better than that of the preparation of an appropriate sample. In this review of the technique, we will focus on several examples, namely a photovoltaic thin film, a biocompatible film coating, and a biomimetic lipid bilayer membrane system to demonstrate the power of the method. We will show how a direct inversion is performed as well as an indirect alternative involving simultaneous fitting of the same two composite system (“unknown film of interest” plus given reference) reflectivity data sets (employed in the direct inversion). We will also consider methods for determining uncertainties in the structural parameters used to describe the SLD depth profile associated with the reflectivity data. All of the programs applied in the paper are publicly available on the web [38].

2. Analysis of PSNR measurements of a photovoltaic film: variation of backing medium

As a first example of how phase-sensitive neutron reflectometry can be applied to study a film system of current scientific and technical interest, consider the organic photovoltaic (PV) film system investigated by Jon Kiel et al. and originally reported on in [39,40].

In polymer based solar cells, device performance is largely determined by the morphology of the active layer components on a length scale of nanometers. However, structural characterization by X-rays is difficult because the constituent materials typically have exceedingly low electron contrast relative to one another. On the other hand, PSNR is well-suited for characterizing the cross-sectional profiles of these bulk hetero-junction (BHJ) systems due to the significant SLD difference between two common device materials, poly(3-hexylthiophene) (P3HT) and [6,6]-phenyl-C61-butyric acid methyl ester (PCBM). In the studies by Kiel et al. [39,40], it was conclusively shown, using PSNR, that the PCBM concentration was significantly enhanced at the interface between film and substrate and also near the air interface. This information is difficult, if not impossible, to obtain by any other means.

To provide the reference structure for the PSNR measurements on the photovoltaic film system, the backing medium, namely an aqueous reservoir, was employed. Reflectivity data sets for two composite systems, i.e., photovoltaic film plus surrounding media, were measured. In both cases, the fronting medium consisted of single crystal line Si, including the substrate on which the photovoltaic film was deposited, whereas the backing medium was defined by a cylindrical volume in a cell which could be filled with either gas or fluid, with a thickness of about 100 μm. In one case this backing volume was air, in the other D₂O. Fig. 1 shows a schematic of the sample configuration and scattering geometry.

Reflectivity data for the composite system consisting of Si fronting medium, sample film, and D₂O backing medium is plotted at the top of Fig. 2. Similarly, the reflectivity obtained with the same sample film and fronting medium but with air as the backing is shown at the bottom of Fig. 2.

2.1. Analysis via direct inversion

From these two composite system reflectivity data sets, the real part of the complex reflection amplitude, Re r(Q), corresponding to the SLD profile of the sample film alone was extracted through an exact solution of a set of linear simultaneous equations [19,31] and is plotted in Fig. 3. The imaginary part of the complex reflection

![Fig. 1. Schematic of fluids cell used for the neutron reflectivity study of the photovoltaic film described in the text. In this configuration the incident and reflected beams traverse the “fronting” medium which includes the substrate (also Si in this case). The Si is in perfect single crystal form so that the net attenuation of the beam through a typical distance of 7.5 cm is only about 15%. The transverse coherence length of a neutron wave packet is of the order of a micron as prepared in a typical neutron reflectometer. At small glancing angles of incidence, the projection of this length in the plane of the film can be tens or even hundreds of microns. On the other hand, its projection along the normal to the sample film is relatively small (over the range of incident glancing angles covered in a specular scan) and thus precludes coherent interaction with material on the other side of the fluids reservoir which is approximately 100 μm thick. Other, nonspecular and frequently incoherent scattering from the reservoir and material beneath contributes a background which is subtracted from the coherent specular signal. A fluids cell such as this allows specular NR measurements to be performed on a film system in intimate contact with a fluid reservoir where, for example, the fluid can be changed in situ. This cell can also be adapted with appropriate electrodes so that electrical potentials can be applied. (From Fig. 2 of Kiel et al. [39]).]
amplitude, \( \text{Im} r(Q) \), was also retrieved simultaneously in the process. As discussed in detail in \cite{31} and specifically in relation to the photovoltaic film investigated by Kiel et al. \cite{39,40}, \( \text{Im} r(Q) \) can serve as a powerful diagnostic of the effectiveness of the incident neutron wave packets in coherently averaging over any in-plane SLD inhomogeneities that might be present. This can be of crucial importance if the possibility exists for in-plane domains of different SLD to be of great enough dimensions that the neutron “sees” one such region independently of another adjacent region. If so, then the measured reflectivity will consist of an area-weighted incoherent sum of separate reflectivities and must be properly analyzed as such. Applying a first-principles direct inversion of this \( \text{Re} r(Q) \) yields the SLD depth profile with a spatial resolution of the order of 10 to 20 nm as shown in Fig. 4. The concentration of the higher density PCBM material at the interfaces is clearly evident. New programs for performing these two steps in the phase-sensitive analysis of such reflectivity data are freely available through the NIST Center for Neutron Research \cite{38}. With an intuitive graphical user interface, the program DiReFF can be employed to perform phase reconstruction via surround variation \cite{19} and direct inversion \cite{20,31}, thereby yielding the SLD profile from a pair of measured composite system reflectivity curves. In addition to the new fitting, phase determination, and inversion programs, related analytical tools such as Monte Carlo re-sampling \cite{41} are now available at the NCNR which may further aid the analysis of NR data.

If \( \text{Re} r(Q) \) is reconstructed from the SLD profile deduced by this phase-sensitive analysis of the composite system NR data, it may be found to differ to some extent from the input \( \text{Re} r(Q) \) originally obtained from the measured reflectivity data. There are a number of possible reasons for any such discrepancy. First, it must be realized that the one-to-one mathematical correspondence between the specular reflection amplitude and the SLD depth profile is “exact” only given an unlimited Q-range over which the reflectivity data is collected and for statistical uncertainties in the reflectivity data values approaching zero. The connection between the resulting uncertainty in the computed SLD values as a consequence of the aforementioned truncation and counting uncertainties in the reflectivity data has been rigorously established \cite{30}. It was demonstrated in the original work by Kiel et al. \cite{39,40} that any artifacts introduced by truncation and statistical uncertainty were negligible in comparison to the features of the SLD profile near the two interfaces that had been identified as enhanced concentrations of PCBM. The differences between “input” and “output” \( \text{Re} r(Q) \) due to these two causes can be reduced to some degree by an iterative refinement process such as that based on a Newton–Raphson algorithm \cite{42,43}. Nonetheless, with truncated and statistically uncertain data, a certain amount of intrinsic uncertainty in the SLD profile, however small, will always remain.

---

**Fig. 2.** Scaled neutron reflectivity data (symbols) as a function of wave vector transfer Q for composite systems consisting of fronting medium, common photovoltaic film, and variable backing medium. The backing is deuterated water in the top plot and air in the bottom plot. The statistical quality of the NR data with the air backing is not as good as that with deuterated water for the backing medium because the water enhances the net signal strength. The simultaneous fits (solid lines) are also shown. Re-plotting of the original data reported in Kiel et al. \cite{39,40}.

**Fig. 3.** Real part of the complex reflection amplitude \( \text{Re}\{R\} = \text{Re} r(Q) \) corresponding to the common photovoltaic film segment as obtained from the composite NR data of Fig. 2 and as described in the text. Re-plotting of the original result reported in Kiel et al. \cite{39,40}.

**Fig. 4.** Scattering length density (SLD) depth profile across the photovoltaic film as obtained from a direct, first-principles inversion of the \( \text{Re} r(Q) \) shown in Fig. 3. Re-plotting of the original result reported on in Kiel et al. \cite{39,40}.

**Fig. 5.** SLD depth profile as obtained by simultaneous fitting of the same two composite system reflectivity data sets used in the direct inversion. The right-hand vertical scale is meant to cover from just below 1.0 to just above \( 3.5 \times 10^{-6} \text{Å}^{-2} \). The SLD profile of Fig. 4 obtained by direct inversion is plotted as well for comparison. Overall agreement of the two results is good to within the differences arising from truncation and statistical uncertainty in the NR data sets and other systematic uncertainties as discussed in the text. Note that the main result, namely a significantly increased SLD at the two interfaces of the film with its surrounding media corresponding to an enhanced concentration of PCBM, is clearly evident in both profiles. The inset is a pictorial representation of the corresponding compositional distribution across the thickness of the PV film. Figure after the results originally reported by Kiel et al. \cite{39,40}.
However, another, more insidious source of uncertainty can arise from the physical properties of the sample film itself. In the present example, there is the possibility that the initial presumption that water molecules from the aqueous D$_2$O reservoir do not penetrate into the photovoltaic film is not strictly valid. That the initially “unknown” part of the composite structure, in this case the photovoltaic “film of interest”, remain unchanged in the process of varying the neighboring reference medium, namely the D$_2$O/air backing, is, in principle, a fundamental requirement. We will return, in the subsection after next, to further discuss the degree to which this requirement must be fulfilled in practice. In the meantime, we will first consider an alternative to performing the direct inversion.

2.2. Analysis via simultaneous fitting

For a number of reasons it is sometimes advantageous to simultaneously fit the pair of composite system reflectivity data sets described in the preceding section instead of, or in addition to, performing a direct inversion. It can be shown [43] that since both approaches, inversion and simultaneous fitting, employ the same two composite reflectivity data sets, the phase information content ensures an identical and unique result to within that allowed by the inherent uncertainties due to truncation and counting statistics, as discussed earlier. However, the inversion result requires essentially no input parameters and is nearly immediate whereas the simultaneous fitting is not necessarily as efficient.

One of the reasons, for example, that simultaneous fitting can be preferable to direct inversion has to do with the practical difficulty in discerning the physical root of a quadratic equation which can arise in the analysis of the two sets of reflectivity data where a saturated ferromagnetic film of finite thickness (in conjunction with polarized neutron beams) is employed as an adjacent reference structure [43]. (An example of such an application is given in a following section as a third example.) Simultaneous fitting may also be advantageous if the system SLD depth profile can be modeled in terms of appropriate physical parameters or variables.

In the original analysis of Kiel et al. [39,40], in addition to the direct inversion, a simultaneous fitting of the NR data for the photovoltaic films was performed with the GARRe$^2$ reflectivity analysis software developed at the NCNR [38]. (See the Appendix A for more technical detail on the GARRe$^2$ program.) In Fig. 5 is plotted the SLD profile as computed by the simultaneous fitting method, as originally reported by Kiel, et al. [39,40]. The simultaneous fitting was performed with the applied constraint that the SLD profile for the PV film be exactly the same in both composite systems, the one with the air and the other with the D$_2$O backing medium. For comparison, the SLD profile from the original inversion shown in Fig. 4 is plotted as well. Within the uncertainties due to truncation of and noise in the NR data, the two profiles are consistent with one another.

For a multilayer sample with some known structure (i.e. layer “A”, layer “B”, etc.), it is convenient to model specular NR data in terms of slabs that correspond to individual layers. However, no such layer structure can be necessarily assumed a priori for this bulk heterojunction (BHJ), which consists of a low scattering length density P3HT polymer film with an (initially) unknown distribution of high scattering length density PCBM nano-particles. This sort of continuously varying SLD profile can certainly be modeled with discrete slabs, with the user adding layers (and thereby degrees of freedom) to the model until a suitable SLD distribution (and thereby a suitable fit) is achieved. However, the process is at best awkward, as discrete layers are being used to describe what is most likely a continuously varying profile.

Alternatively, free-form modeling with control points connected by splines, can be highly advantageous for samples with no rigid layer structure [27]. Such a free-form model was used to reanalyze the original data obtained for the PV film studied by Kiel et al. [39,40], which we have been discussing here, using the new Refl1D specular reflectivity modeling software [38] (for technical details, see the Appendix A). Except for the SLD of the reservoir media, the models used to fit the air and D$_2$O data were constrained to be identical, with the BHJ represented by six control points connected by linear splines. Thirteen parameters were allowed to vary freely: the total thickness of the BHJ, the scattering length densities of the control points, the relative positions of the interior control points in z (two of the points were constrained to be “endpoints”), the interferential transition width between the BHJ and the reservoir, and the transitional width between the BHJ and the Si substrate. To allow for Gaussian interfacial transitions, each endpoint was adjacent to a narrow (fixed to be three times the interface width) slab layer, with a SLD that matched the corresponding endpoint. Using a combination of fitting algorithms available in the Refl1D suite, reasonably good fits to the data were achieved, as shown in Fig. 6. The corresponding model, shown in Fig. 7 is qualitatively similar to that determined from slab modeling and inversion [39,40], with increased SLD at both ends of the film, corresponding to increased PCBM concentration.

2.3. Analysis via a more independent fitting strategy

Let us now return to the issue, raised in the subsection before last on direct inversion, regarding the effects of an inadvertent change in the SLD profile of the PV film of interest during the course of measuring the NR of the two composite systems. A possible mechanism for such

---

**Fig. 6.** Original composite system NR data (symbols) of Fig. 2 but with fits (lines) generated by the new fitting procedure and program described in the text. A different scaling of the reflectivity has been used in these plots — instead of multiplying the reflectivity by the fourth power of Q, the reflectivity is divided by that for the corresponding semi-infinite fronting and backing media without the PV film (the latter is commonly referred to as the Fresnel reflectivity).

**Fig. 7.** SLD profile for the PV film corresponding to the NR fits shown in Fig. 6 as obtained with the new procedure and program described in the text. The new fitting procedure effectively imposes somewhat different constraints than those of the original simultaneous fitting that yielded the SLD profile of Fig. 5. Nonetheless, the primary result that an enhanced PCBM concentration occurs at the two film interfaces is again clearly manifest.
a change could conceivably involve diffusion of water into any voids that might have been produced in the PV film during the spin-coating deposition process as solvent evaporated. Upon subsequent exposure to the D$_2$O aqueous reservoir, which is the reference backing medium in one of the two composite systems required for the PSNR method, some amount of D$_2$O might have diffused into such voids. Although a number of successive NR scans were performed to check for this possibility, the practical minimum time scale for repeating a complete scan was of the order of hours. Thus, if diffusion occurred in a sufficiently short enough time period, it could go unnoticed.

To investigate the possibility that water had diffused at a faster rate, another simultaneous fit of the two composite system reflectivity data sets for the PV film has been performed here (using the same GARefl program used originally), but with different constraints imposed. Instead of requiring that the SLD profile segment associated with the PV film remain exactly constant in the two composite systems, a less restrictive condition was applied. Specifically, this condition was that a given constant fraction of a presumed void volume in the film contain air in one composite film system and D$_2$O in the other.
The results of such an analysis are displayed in Fig. 8. The fits of the two composite system NR data sets are somewhat better (according to lower chi-squared values) than those obtained with the more fully constrained fits shown before in Fig. 2. Although the relative shape of the SLD profile is nearly the same as that obtained in the original work of Kiel et al. [39,40] – especially concerning the pronounced SLD at the two interfaces due to an enhanced concentration of PCBM – there is a detectable overall shift in the SLD level consistent with a model where diffusion of some D$_2$O occurs in the composite system with D$_2$O in the adjacent reservoir. Although this is in no way affects the original conclusion of the experiment regarding the morphology of the film corresponding to the relative distribution of its P3HT and PCBM components, it does illustrate the need to recognize the possibility of diffusion in film systems where a solvent is used as one or both of the variable reference media. In the next two sections, we will present examples of PSNR wherein other types of references are employed that circumvent this potential complication.

3. Bio-compatible films: variation of fronting medium

The second example of a PSNR study involves a cell membrane mimic system originally reported on by Perez-Salas et al. [44]. Lipid membranes are of general interest because of the important role they play in mediating many biological processes on the cellular level. Supported cell membrane mimics are of specific practical interest because they enable functionalization of inorganic materials by creating bio-compatible surfaces – e.g., coatings for artificial organs or other implantable objects such as synthetic veins and arteries. This three part system was made up of a polyelectrolyte multilayer, synthetic terpolymer, and phospholipid layer (PE+TER+PC) all deposited, sequentially, on a solid support substrate – a schematic is shown in Fig. 9. The polyelectrolyte layer acts as a water permeable cushion on which the terpolymer and phospholipid layers are supported. The distribution of water across the thickness of the membrane layer system was obtained via PSNR.

In this case, to enable PSNR, two support media were used, one a single crystal of Si, the other of Al$_2$O$_3$. By performing NR measurements on these two composite systems, Si plus membrane and Al$_2$O$_3$ plus membrane, PSNR was possible via variation of the fronting substrate medium [19]. (In both of these composite systems, the substrate was first coated with a Au layer approximately 80 Å thick. This allows the polyelectrolyte layer of the common film of interest to bind to an identical surface on the two different substrate or fronting materials that serve as the references.) In this set-up, the membrane could be exposed to a variety of humid atmospheres at the backing interface and PSNR could be performed for each of these conditions by collecting NR data for both composite systems. Unlike the previous PV film example, reference variation does not affect the film of interest. However, it was necessary to ensure that the same membrane system was deposited on both the Si and Al$_2$O$_3$ substrates. As mentioned above, the distribution of water across the thickness of the membrane

![Fig. 10. Re r(Q) for (a) (PE+TER) sub-assembly and (b) the full (PE+TER+PC) film assembly in 100% deuterated water at 92% humidity. The symbols are for the values computed from the two composite system NR data sets whereas the lines are the values reconstructed from the corresponding SLD depth profiles obtained by direct inversion. The close agreement is indicative of a high degree of self-consistency.](image)

![Fig. 11. (a) SLD depth profiles for the (PE + TER + PC) film assembly in 100% D$_2$O (dashed line) and 50%/50% D$_2$O/H$_2$O (solid line) at 92% humidity. (b) Water fraction across the film assembly as deduced from the two isotopic mixtures. By performing the isotopic variation, it is possible in this case to directly obtain a compositional depth profile for the water. In other cases, molecular dynamics simulations have proven valuable in relating neutron SLD and composition depth profiles obtained by NR [45].](image)
layer system was obtained. This was accomplished using PSNR methods and different D₂O and H₂O mixtures in the backing volume. Note that in this approach, PSNR is performed via variation of the fronting medium (Si or Al₂O₃) for each H₂O and D₂O mixture.

Fig. 10 shows the real part of the complex reflection amplitude for the (PE + TER + PC) membrane (including the Cr/Au metal underlayer as part of the SLD profile of the “unknown” film of interest being solved for) in 100% D₂O at 92% humidity — as deduced from the two composite system NR data sets corresponding to membrane on Si or Al₂O₃ fronting substrate (symbols). In addition to the symbols representing Re r(Q) obtained point-by-point from the two composite system reflectivity data sets, the line plotted in Fig. 10 is that calculated or reconstructed from the SLD profile obtained by direct inversion of the Re r(Q) from the original data. The agreement is excellent and demonstrates a high degree of self consistency in the phase determination/inversion process.

Fig. 11 plots the SLD profiles for the (PE + TER + PC) membrane in 100% D₂O at 92% humidity (small dashed line) and also, corresponding to another two sets of composite system NR data, the profile for the case of 50% D₂O/50% H₂O mixture at 92% humidity (solid line). By analyzing the SLD profiles for different water mixtures and humidities, it was possible to ascertain the distribution of water itself across the thickness of the membrane system as is shown in Fig. 11b. The water fraction peaks at approximately 40% uptake in the polyelectrolyte layer (PE) which is closest to the Au layer on the supporting substrate. The spatial resolution is of the order of a nanometer and, once again, illustrates the power of the PSNR technique for studying the composition of organic film systems. Nonetheless, preparing the membrane system reproducibly and consistently on the two different support substrates of Si and Al₂O₃ necessary to conduct the measurements is demanding and for other systems might not be practically feasible. As alluded to earlier, an alternative reference structure employs a buried magnetic layer on a single supporting substrate which can be tuned to two different SLD values if polarized neutron beams are used to perform the reflectivity measurements. An example of the application of such a reference structure is given in the next section.


In a series of articles, Le Brun and Holt et al. demonstrated how PSNR could be practically applied to the study of biomembranes involving antibody-binding membrane protein arrays [46], ion-channels [47], and engineered biosensor surfaces [48]. Here we will focus on the study of the ion-channel-containing model membrane and the determination of its structure along the membrane normal [47]. For a critique of NR studies of other biomembrane systems, see the reviews by Krueger and Wacklin [7,9], or for a recent specific illustrative example, see, for instance, reference [49].

Specular PSNR is, arguably, the most sensitive method currently available for determining the positions of separate organic molecular components along the normal of a single bilayer with a fraction of a nanometer spatial resolution — and at the same time allowing in situ manipulation of the sample system and its aqueous environment [7]. The system investigated by Holt et al. [47] consists, essentially, of an outer membrane protein from Escherichia coli, OmpF, which is one of a large group of barrel membrane proteins, adsorbed onto some fraction of the area of the gold surface and surrounded by a lipid membrane containing thiols which adsorbed to the remaining gold surface area. The self-assembly of this complex membrane structure was monitored sequentially through the various stages of the deposition processes, as detailed in the original paper [47], with SLD profiles determined by PSNR. Fig. 12 shows a schematic picture of the system.

PSNR methods were enabled through the use of a saturated ferromagnetic layer buried beneath an Au layer on a Si substrate upon which was deposited the biomembrane in an aqueous environment. The Si substrate served as the fronting medium. Using a polarized neutron beam, one of the two spin eigenstates (+ or −) encounters a SLD in the magnetic layer that is the sum of nuclear and magnetic SLD and the other a SLD that is the difference between the two. By performing two composite system NR measurements, one for a beam in the + spin state and the other in the − spin state, PSNR is possible [34,37]. A variety of magnetic layer materials were employed, including pure Fe, “mu” metal, and an FeNi alloy (approximately 20% Fe and 80% Ni) known commercially as Permalloy. Simultaneous fitting of the two composite system reflectivity data sets was employed using the GARf program described in a previous section.

Fig. 13a shows the neutron reflectivity data (for one of the two spin states) at various stages in the deposition of the film system, represented pictorially in Fig. 12, whereas the corresponding SLD depth profiles are shown in Fig. 13b. To further quantify the accuracy of the results for the profile thus obtained by PSNR methods, a Monte-Carlo based resampling technique [41] was applied. One
example of this analysis is shown in Fig. 14 for the most probable thickness and SLD values of the self-assembled OmpF layers. The same analysis was applied to the other components of the complete membrane system. This study by Holt et al. [47] demonstrates the power and sensitivity of PSNR coupled with other analytical tools, such as Monte-Carlo re-sampling, when applied to sample systems prepared with commensurate quality—detailed information about the cross-sectional structure of such a membrane system can be obtained thereby with nanometer scale spatial resolution.

5. Conclusion

Phase-sensitive neutron reflectometry (PSNR) is a technique that is especially well-suited for studying the molecular scale structure of layered thin film materials. In particular, specular PSNR, in which the momentum and wavevector transfer is normal to the surface, can accurately reveal detailed features of the compositional depth profile with a spatial resolution approaching a fraction of a nanometer under proper conditions. Although specular PSNR is fundamentally a diffraction method, the preservation of phase information eliminates the characteristic ambiguity intrinsic to ordinary diffraction and enables unique structural solutions that, in essence, are equivalent to real space images. Moreover, for organic soft matter, PSNR can complement X-ray reflectivity measurements because of the neutron’s particular sensitivity to hydrogen and deuterium.

Acknowledgments

We would like to acknowledge our many colleagues with whom we have worked performing neutron reflectometry studies of soft matter systems over the years and who have contributed greatly to this research.

A number of key translations of code written in a variety of different computer languages to Python were performed. Pikaia was translated from FORTRAN by Mathieu Doucet; Snobfit was translated from Matlab.
and GEPORE was translated from FORTRAN by Ziwen Fu; Particle Swarm and Random Lines were translated from Matlab by Ismet Sahin; Mlayer and gj2 were translated from FORTRAN by Kevin O’Donovan; graphical user interfaces for Reff1D and DiRefl are in part provided by James Krycka and Nikunj Patel. Additional software was provided by Wenwu Chen, Alexander Mont and David Tighe.

Appendix A

The following two applications run on both Windows and Linux.

A.1. GAREfl Program

The mlayer program [50] provides a simple layer model and a Levenberg–Marquardt [51] refinement algorithm. Layer interfaces follow an erf or tanh profile, represented by a fixed number of slabs. The slab widths are chosen such that the change in SDL is fixed between each pair of slabs. Constraints are not handled by the refinement algorithm, but are instead provided by a user defined constraints function which rewrites the parameter values before creating the reflectivity profile. The gj2 program adds support for polarized neutron reflectometry. Relfpak provides a graphical user interface around mlayer and gj2.

The program GAREfl uses the layer model representation of mlayer and gj2, but allows simultaneous refinement of multiple data sets, and incorporation mixing of multiple reflectivity models per data set. Gaussian interfaces can be approximated analytically [52] or by using multiple slabs. GAREfl adds the Nelder–Mead local refinement algorithm, Amoeba [51], and a genetic algorithm, Pikaia [53] for global refinement. Amoeba and Pikaia allow bounds constraints on the parameters. More complex constraints are again provided by a user defined constraints function which rewrites the parameter values. Uncertainty analysis is performed using bootstrap or re-sampling Monte Carlo which rewrites the parameter values. Uncertainty analysis is performed using bootstrap or re-sampling Monte Carlo which rewrites the parameter values before creating the reflectometry. Pikaia is replaced with a set of global re

A.2. Reff1D program

Reff1D adds freeform profiles and interfaces and user defined profiles. An important feature of Reff1D is its intuitive graphical interface (GUI). Freeform profiles and interfaces are based on monotone cubic splines [54]. With monotone splines, the profile between two control points is guaranteed to lie between the control points. Thus the user can limit the scattering potential of a region to the known scattering potential of the materials in the sample without limiting how those materials are distributed. Furthermore, the control points can be constrained to be monotonic, thus providing a convenient representation of an unknown but physically plausible diffuse interface. To efficiently compute the reflectivity, non-uniform profiles are computed on a fine mesh, with neighboring slabs coalesced if the scattering potentials are similar. Pikaia is replaced with a set of global refinement algorithms: multistart amoeba, random lines [55], differential evolution [56], parallel tempering [57], particle swarm [58], snobfit [59]. Model parameters in Reff1D can be tied directly to each other, eliminating the need for the user to provide a constraints function. Instead of minimizing chi2, the optimization problem is recast as minimizing the negative log likelihood function. This allows the systematic inclusion of prior information about the system such as the estimated uncertainty in the sample alignment as measured by the rocking curve. Parameter uncertainty is estimated using Markov chain Monte Carlo analysis [60]. Because the probability density function is poorly conditioned, simple MCMC update algorithms are inefficient, and we instead use the DiffEential Evolution Adaptive Metropolis algorithm (DREAM) [61].

References


