UNIQUE DETERMINATION OF BIOMIMETIC MEMBRANE PROFILES BY NEUTRON REFLECTIVITY

New biomimetic membrane materials, of fundamental importance in understanding such key biological processes as molecular recognition, conformational changes, and molecular self-assembly, can be characterized using neutron reflectometry. In particular, scattering length density (SLD) depth profiles along the normal to the surface of a model biological bilayer, which mimics the structure and function of a genuine cell membrane, can be deduced from specular neutron reflectivity data collected as a function of wavevector transfer Q. Specifically, this depth profile can be obtained by numerically fitting a computed to a measured reflectivity. The profile generating the best fitting reflectivity curve can then be compared to cross-sectional slices of the film's chemical composition predicted, for example, by molecular dynamics simulations [1]. However, the uniqueness of a profile obtained by conventional analysis of the film's reflectivity alone cannot be established definitively without additional information. In practice, significantly different SLD profiles have been shown to yield calculated reflectivity curves with essentially equivalent goodness-of-fit to measured data [2], as illustrated in Fig. 1.

The existence of multiple solutions, only one of which can be physical, is especially problematic in cases where a key additional piece of structural or compositional information is lacking as can happen in the investigation of these biological membrane systems.

Why this inherent uncertainty? The neutron specular reflection amplitude for a model SLD can be computed exactly from first principles; the square of its modulus gives the measurable reflectivity. It is firmly established, however, that the complex amplitude is necessary and sufficient for a unique solution of the inverse problem, that of recovering the SLD from reflection measurements. Unambiguous inversion requires both the magnitude and phase of reflection. Once these are known, practical methods [3] exist for extracting the desired SLD.

In fact, considerable efforts were made about a quarter century ago to solve the analogous "phase problem" in X-ray crystallography using known constraints on the scattering electron density [4] and by the technique of isomorphic substitution [5]. Variations of the latter approach have been applied to reflectivity, using a known reference layer in a composite film in place of atomic substitutions. These

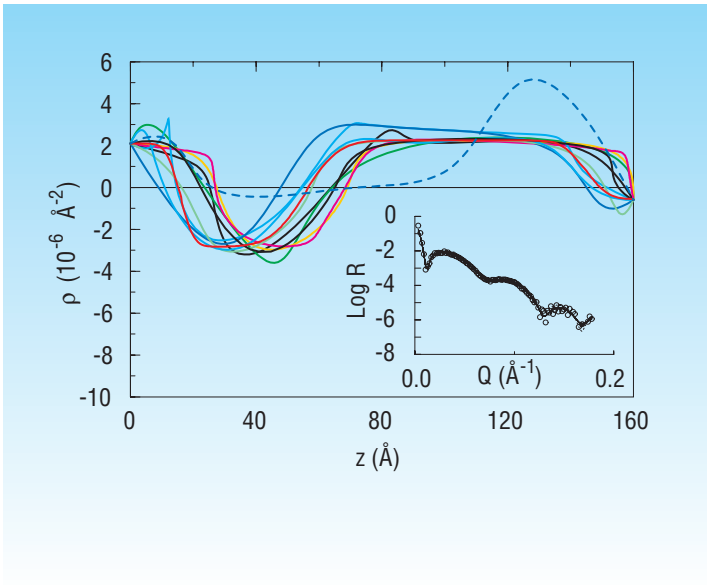

FIGURE 1. Family of scattering length density profiles obtained by model-independent fitting of the reflectivity data in the inset. The profile represented by the blue dashed line is unphysical for this Ti/TiO film system yet generates a reflectivity curve that fits the data with essentially equivalent goodness-of-fit (all the reflectivity curves corresponding to the SLD's shown are plotted in the inset but are practically indistinguishable from one another).

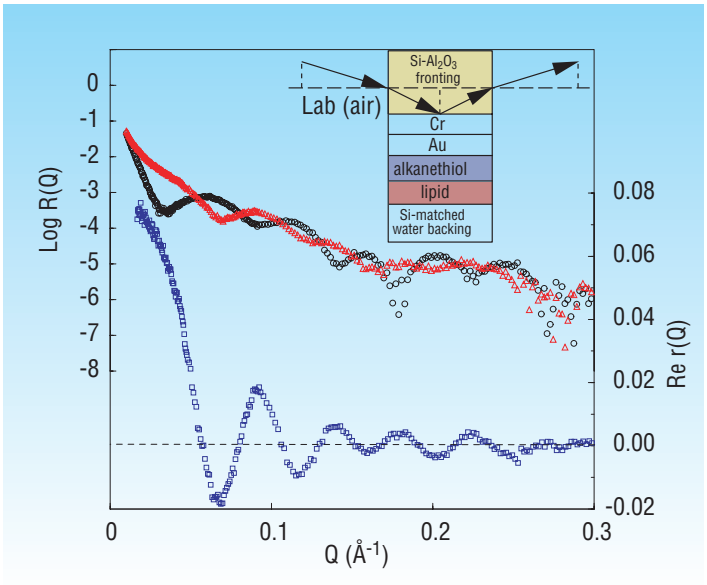

FIGURE 2. Reflectivity curves for the thin film system depicted schematically in the inset, one for a Si fronting (red triangles), the other for Al_2O_3 (black circles). The curve in the lower part of the figure (blue squares) is the real part of the complex reflection amplitude for the films obtained from the reflectivity curves by the method described in the text.

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solution methods, however, were tied to the Born approximation, which generally is valid in crystal structure determination but which fails catastrophically at low Q (low glancing angles) in reflection from slab-shaped samples such as thin films. Exact inversion requires accurate knowledge of the reflection amplitude over the entire Q-range, especially at low Q.

In this decade the reflection phase problem has been exactly solved using a protocol of three reflectivity measurements on composite films consisting of the film of interest in intimate contact with each of three known reference layers [6, 7]. Subsequently, variations using only two measurements have been shown to partially solve the phase problem, an additional procedure being required to choose between two solution branches, only one of which is physical [8, 9]. In the past year [10], an exact solution has been found for a two measurement strategy in which the film surround, either the fronting (incident) or backing (transmitting) medium, is varied. This new approach is simpler to apply than reference layer methods and is adaptable to many experiments. Surround variation neutron reflectometry has been successfully applied to the challenging type of biological membrane depth profiling described earlier.

In Fig. 2 are plotted a pair of neutron reflectivity curves measured for the layered film structure schematically depicted in the upper right inset, one with Si and the other with Al_2O_3 as the fronting medium. The lower part of Fig. 2 shows the real part of the complex reflection amplitude for the multilayer as extracted from the reflectivity data, according to the method described above, and which was subsequently used to perform the inversion to obtain the SLD shown in Fig. 3. For comparison, the SLD predicted by a molecular dynamics simulation is also shown in Fig. 3, in a slightly distorted version, corresponding to a truncated reflectivity data set, which indicates the spatial resolution of an SLD obtainable in practice. This latter SLD was obtained by inversion of the reflection amplitude computed for the exact model SLD, but using values only up to the same maximum Q value (0.3 $Å^{-1}$) over which the actual reflectivity data sets were collected. Overall, agreement between the experimentally determined profile and the theoretical prediction is remarkable, essentially limited only by the Q-range of the measurement. Surround variation neutron reflectivity thus makes it possible to measure complicated thin film structures without the ambiguity associated with curve fitting. The veridical SLD profile is obtained directly by a first principles inversion.

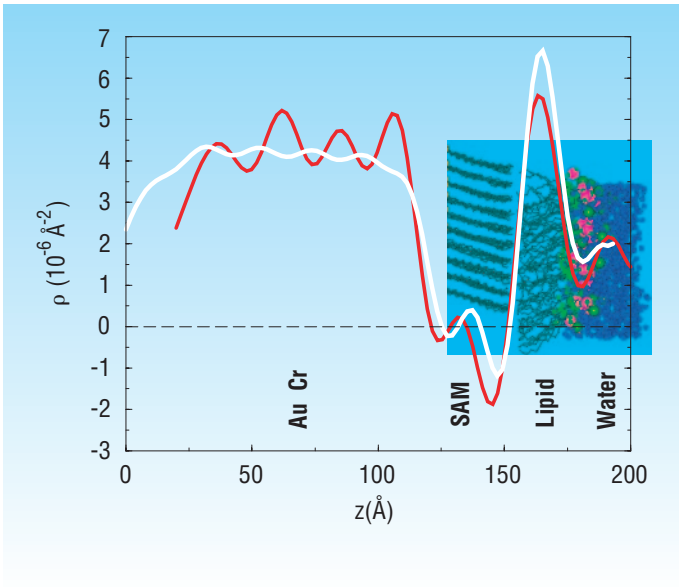

FIGURE 3. SLD profile (red line) resulting from a direct inversion of the Re r of Fig. 2 compared with that predicted by a molecular dynamics simulation (white line) as discussed in the text. The headgroup for the Self-Assembled-Monolayer (SAM) at the Au surface in the actual experiment was ethylene oxide and was not included in the simulation but, rather, modelled separately as part of the Au. Also, the Cr-Au layer used in the model happened to be 20 Å thicker than that actually measured in the experiment.

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