CHARACTERIZATION OF NEW BIOMIMETIC MATERIALS USING NEUTRON REFLECTIVITY

All cells are enclosed by a biological membrane, consisting of assemblies of lipid and protein molecules, that defines its boundaries and regulates its interactions with the environment. 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 membranes, which are simpler than cell membranes but mimic their structure and function, are used to study these complicated systems. Such model membranes are known as biomimetic materials, which emulate biological function such as molecular recognition, dynamic conformational change and spontaneous self-assembly of complex arrays of molecules.

A biomimetic material which is analogous to the lipid membranes of cells and can support active membrane proteins has been made in NIST's Biotechnology Division [1]. This hybrid bilayer membrane (HBM), which is illustrated in Fig. 1, consists of two self-assembling monolayers, one which is non-biological (alkanethiol) and a second which can be found in biological cell membranes (phospholipid). This system is formed spontaneously on a planar gold surface. Since the alkanethiol monolayer is strongly bonded to the gold surface, this HBM is more rugged than a conventional supported phospholipid bilayer,

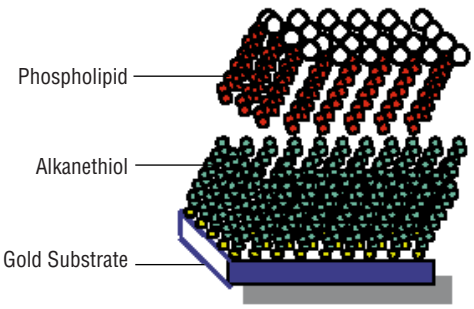

FIGURE 1. The ethyleneoxide-alkanethiol/phospholipid hybrid bilayer membrane (HBM).

FIGURE 2. Members of the experimental group at the NG1 reflectometer at the NCNR. Clockwise from bottom: S. Krueger, A. L. Plant, C. W. Meuse, N. F. Berk and C. F. Majkrzak.

which binds only weakly to a silicon or glass surface. In addition to their obvious importance as a tool for understanding and characterizing membrane protein structure and function, the biomimetic characteristics of the HBMs make them commercially significant for a number of applications including biosensors, tissue engineering, and bioelectronics and biocatalysis. The lipid and protein composition of the HBM can be readily engineered to produce structures with novel physical and chemical properties that do not occur in nature.

The development of measurement tools for probing the structure and function of these engineered membranes and the cell membrane components incorporated into them is essential for the optimization of their biomimetic character. To this end, the neutron reflectivity technique is being used to assist in the structural characterization of HBMs which are in contact with water. Such *in situ* measurements are only possible because neutrons interact weakly with materials, in contrast to electromagnetic probes such as light, x-rays and electrons. Thus, the planar substrate can be used as the incident medium, allowing the phospholipid side of the HBM to be in full contact with water, as it is in its native state. The neutron reflectivity measurements are being made on the NG1 reflectometer at the NCNR, shown in

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Fig. 2. Advancements in instrumentation, sample environment and measurement protocols now make it possible to obtain Angstrom-level information about the composition of HBMs along the axis perpendicular to the plane of the membrane.

The results from recent neutron reflectivity measurements of HBMs in water are shown in Fig.3. The HBMs were formed on single crystal silicon substrates, which had been coated with ~ 50Å of gold on a ~ 15Å chromium adhesion layer, and were measured in contact with water. The neutron scattering length density (SLD) profile shown in Fig. 3 was obtained by fitting the reflectivity data using the model-independent fitting program, PBS [2]. Since the neutron scattering length density of each element in the bilayer depends upon its chemical composition, the SLD profile is essentially a map of the bilayer structure in the plane perpendicular to that of the membrane, generally defined as the Z direction. The silicon substrate is at Z = 0 by definition. The locations of the gold layer, the alkanethiol monolayer and the phospholipid monolayer relative to the substrate can be easily distinguished in the SLD profile.

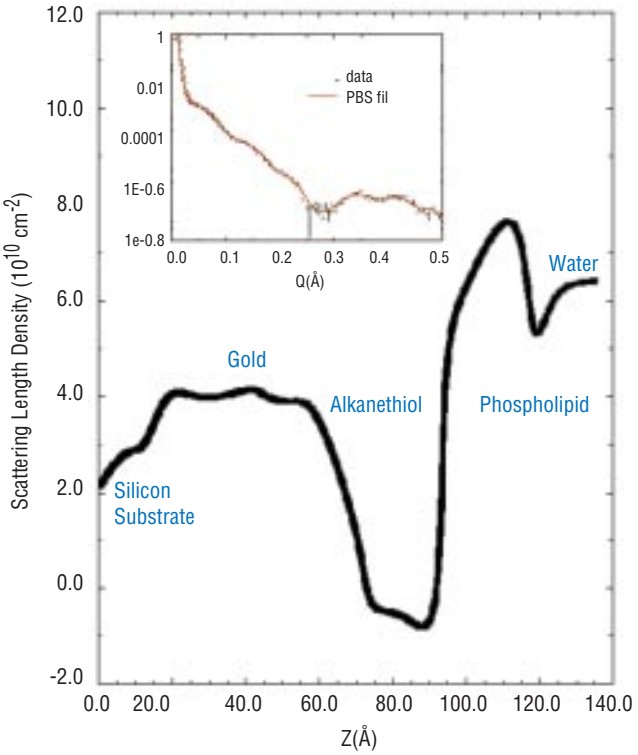

FIGURE 3. A representative neutron scattering length density profile for a hybrid bilayer membrane. The location of the silicon substrate is defined as Z = 0. The corresponding neutron reflectivity data are shown in the inset.

Neutron reflectivity measurements have also been made, for the first time, on HBMs in the presence of the membrane protein, melittin, a relatively small peptide toxin that is found in bee venom. Although melittin is an important model compound for pore-forming peptides such as antibiotics, its exact location in the membrane is not known. The structure of HBMs in the absence and presence of melittin now can be directly compared and questions about how deeply melittin penetrates into the bilayer, and whether melittin forms pores that allow water into the bilayer, can be addressed.

Most recently, neutron reflectivity has been used to study the structure of a novel HBM matrix consisting of an ethyleneoxide-containing alkanethiol monolayer and a phospholipid monolayer. The ethyleneoxide moiety tethered to the gold surface was intended to act as a loosely-packed “spacer”, allowing water to penetrate into the region near the gold surface, thus providing a suitable environment for the incorporation of transmembrane proteins. However, the neutron reflectivity measurements provided direct evidence that the ethyleneoxide region contains no water. Furthermore, the reflectivity measurements confirmed that melittin does not alter the membrane structure in a way that would allow water into this region. These important results have led to the development of new HBMs, which more closely mimic biological membranes and are capable of supporting transmembrane proteins.

REFERENCES

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[2] N. F. Berk, and C. F. Majkrzak, Phys. Rev. B **51**, 11296 (1995).