Neutron Reflectivity investigation of the structure of a phospholipid bilayer membrane and a membrane-associated protein.

Summer School on the Fundamentals of Neutron Scattering
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Introduction

Phospholipid bilayer are the basic structural element of biological membranes. They separate the various cell compartments found in eukaryotic cells and form an outer barrier (cell membrane or plasma membrane) of eukaryotic and prokaryotic cells separating the cytoplasm from the extra-cellular space. The composition of phospholipid bilayers varies with the cell type and function of the membrane. It commonly contains, for example, charged lipids, neutral or zwitterionic lipids, glycolipids, cholesterol, and sphingomyelin. The hydrocarbon core of the lipid bilayer forms a passive barrier for ions, sugars, and other metabolites. The lipid components of the membrane provide a matrix for incorporation of membrane proteins that are involved in cell processes such as transport of ions and molecules across the membrane, cell signaling, and ligand recognition (receptors). Biological phospholipid bilayers are actively kept in a fluid state [Sin72], allowing lipids and membrane proteins to diffuse in the lipid bilayer and to form functional domains for cell processes.

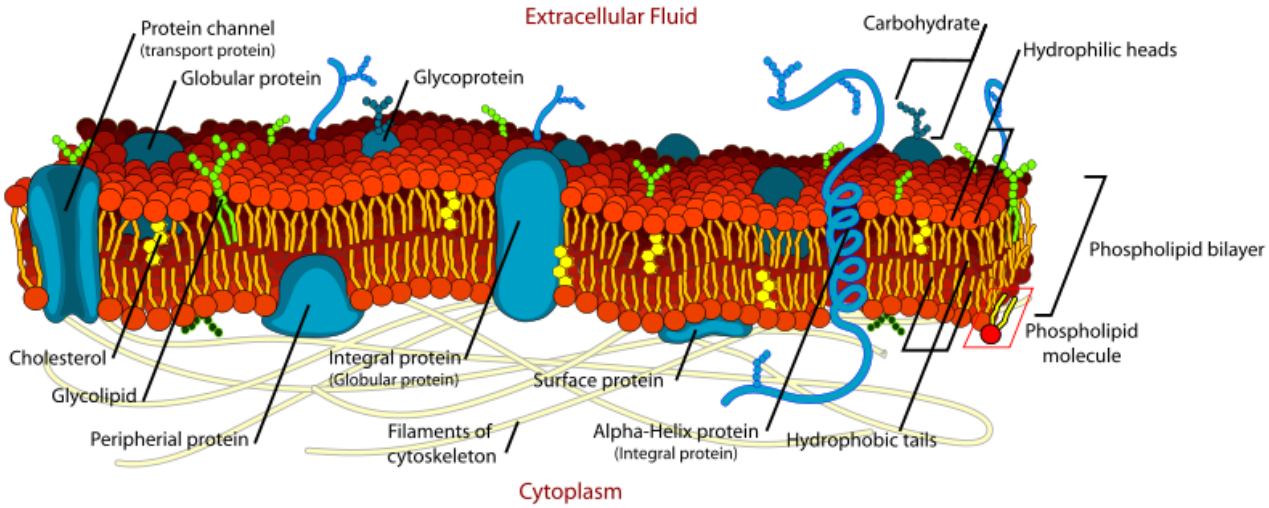


Illustration of a biological cell membrane. From www.wikipedia.org.

For biophysical investigations of a single type of protein with the lipid membrane, a lipid membrane of a biological composition would be far too complex. Instead, well-characterized model membranes of controlled composition and physical properties are employed [Cas06]. For geometrical reasons, model membranes for reflectivity experiments have to be supported by a plane substrate. The simplest model system, which will be structurally characterized during this tutorial experiment, is a lipid bilayer of a single type of

lipid directly deposited onto a silicon wafer. The silicon wafer provides an atomically flat surface and has a comparably low scattering cross-section – two properties that makes it an ideal substrate for reflectometry. The deposited lipid bilayer is separated by the substrate by an approximately 5 Å water gap, and therefore interacting strongly with the substrate. This interaction impairs the fluidity of the lipid leaflet proximate to the substrate. In addition to steric constraints, this also impedes the incorporation of integral membrane proteins. Nevertheless, the described model system can be successfully applied for investigations of the interaction of peripheral proteins with the outer lipid leaflet.

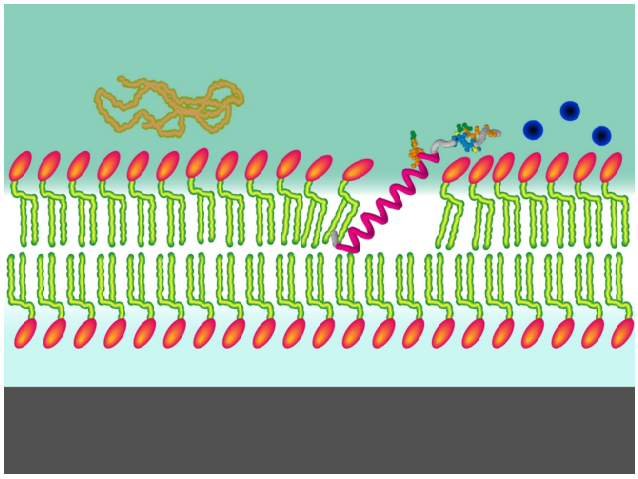


Illustration of a solid supported lipid bilayer membrane interacting with various biomolecules.

More complex model systems, like the tethered lipid bilayer system routinely used at the NCNR [Gil07, Hei09], decouple the lipid bilayer from the solid support using spacer molecules that provide relatively large water-filled sub-membrane space. This model system allows for the incorporation of integral membrane proteins and both lipid leaflets are in the fluid state.

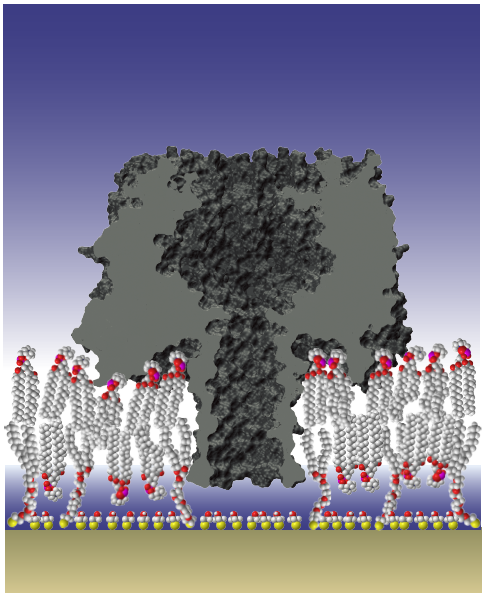


Illustration of a tethered lipid bilayer (tBLM) with a transmembrane pore, as measured using neutron reflectivity at the NCNR [Gil09]. A sparse coverage of tether molecules creates a water-filled sub-membrane space between the lipid bilayer and the substrate which decouples the membrane and the protein from the substrate. The small molecules are called backfiller molecules and regulate the tether density.

Goal of the Tutorial Experiment

This tutorial experiment has the following goals:

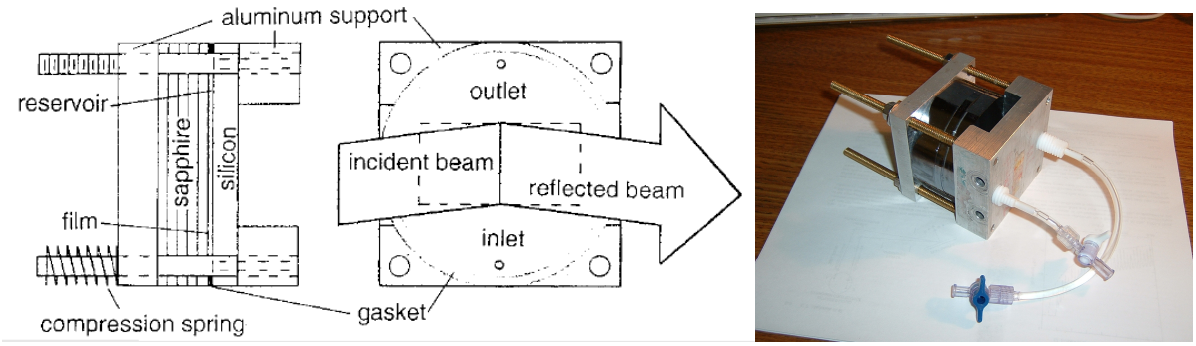
1) Learning about membrane model systems for neutron reflectivity and the various factors that contribute to a successful biological experiment.
2) Structural characterization of tethered lipid bilayer membranes (tBLM) formed on a gold surface. This includes:
    - the characterization of bilayer thickness.
    - the determination of the headgroup hydration
    - the determination of the bilayer completeness
    - the determination of the area per lipid
    - the measurement of the thickness of the hydration layer between the bilayer and the substrate.
3) Adding a protein (PlyCB) to the tBLM and measure protein adsorption. This protein is known to embed either in the hydrocarbon core of the lipid bilayer, or to associate peripherally with the membrane, depending on the lipid composition of the bilayer. The experiment will determine the protein envelope and location using a membrane composed of 70:30 POPC: POPS.
4) Learning about the optimal number of isotopic solvent contrasts per condition to be measured during the biological reflectometry experiment.

Planning of the Experiment and Sample Preparation

Samples of tBLMs are prepared on 3" silicon wafers which were coated with a thin gold film. The lipids used during this experiment are the zwitterionic POPC and the anionic POPS. The completeness of the formed bilayer and thickness of the sub-membrane water gap can be determined from the reflectivity data if the sample is measured in contact with at least two isotopically distinct aqueous buffers, such as D_2O-based and H_2O-based buffer. This is why at least two reflectivity measurements per sample will be carried out and the buffer solution will be exchanged between the measurements.

The sample preparation is a multi-step process. First a self-assembled monolayer (SAM) of tether molecules is formed on the gold surface by adsorption from an ethanolic solution of tether molecules. The deposition of a lipid bilayer onto the SAM will be achieved by vesicle fusion. Here, a dry lipid film is suspended in aqueous buffer and vesicles are formed by ultra-sonication. The SAM is then incubated with vesicles for an extended time. Finally, excess non-fused vesicles are rinsed off the interface. This easy preparation technique usually results in a very complete lipid bilayer formation over large areas.

For neutron reflectivity measurements this preparation is carried out in a wet cell. The NCNR wet cell posses one inlet and one outlet for solvent exchanges. The water reservoir on-top of the prepared film is merely 100 μm thick. After measurement of the as-prepared bilayer, protein will be added in situ, and structural changes will be characterized using at least two isotopic solvent contrasts.

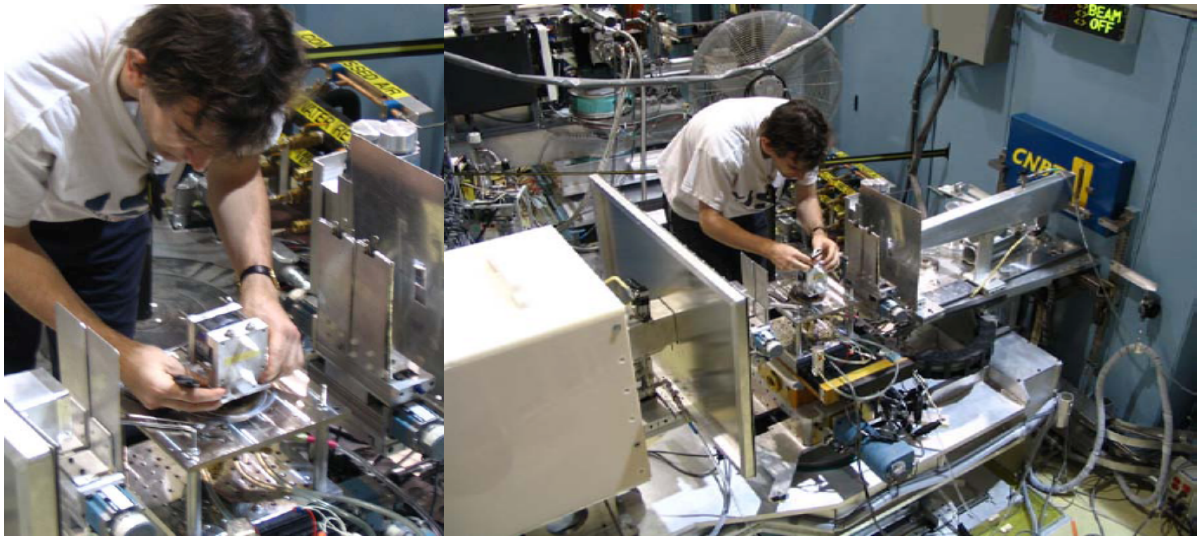


Left: Drawing of the NCNR wet cell [Maj00]. In the drawing the film is prepared on a wafer made of sapphire instead of silicon. Right: Photo of the NCNR wet cell. The wafer with the film is the thin 5 mm wafer in the middle of the sandwich. To the left from it there is the thick 15 mm fronting wafer, to the right there is the thin 7 mm wafer with holes for the buffer exchange inlet and outlet.

Data Collection and Data Reduction

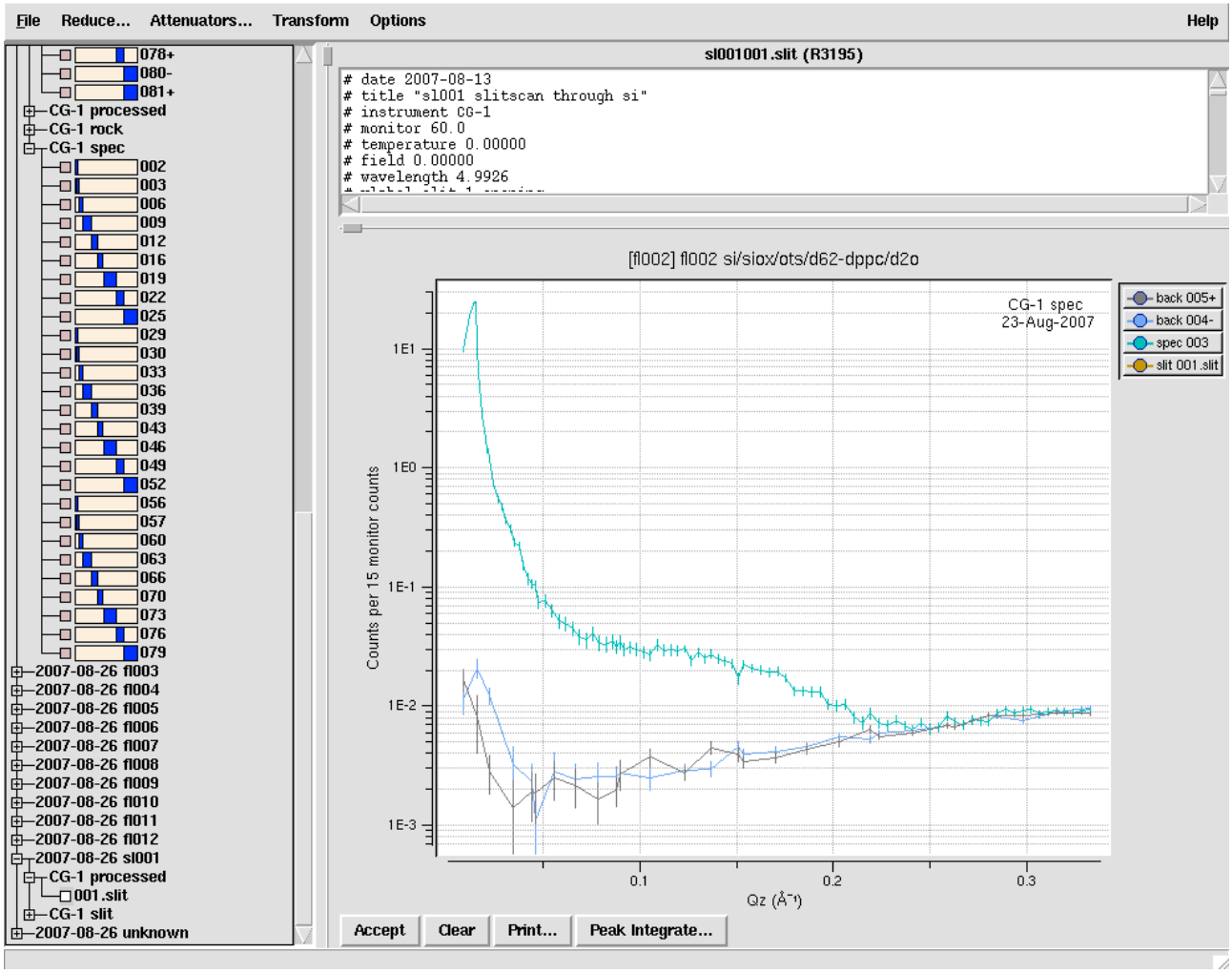
For a detailed description of the neutron reflectometry technique see the excellent tutorial written by Andrew Jackson, posted on the Summer School website.

The prepared wet cell has to be aligned on the instrument. Prior to the neutron reflectivity measurement an incident beam scan through the thick fronting wafer is performed in order to measure the direct beam intensity. The later-on measured specular reflectivity will be normalized to the direct beam incident intensity.



Duncan McGillivray aligns the NCNR wet cell.

The aligned wet cell is first filled with D_2O-based buffer and the specular reflectivity is measured between momentum transfers $0 \leq q_z \leq 0.30$ Å^{-1}. The background intensity offset to both sides of the specular ridge is measured separately. A large contribution to the background neutron radiation originates from incoherent scattering from the bulk solvent reservoir. The measurement is repeated after filling the wet cell with H_2O-based buffer. This way two distinct data sets of the same sample in contact with isotopically different bulk solvents are recorded. The neutron reflectivity is calculated from the measured specular raw data, the background data and the incident beam data. This process is called data reduction.



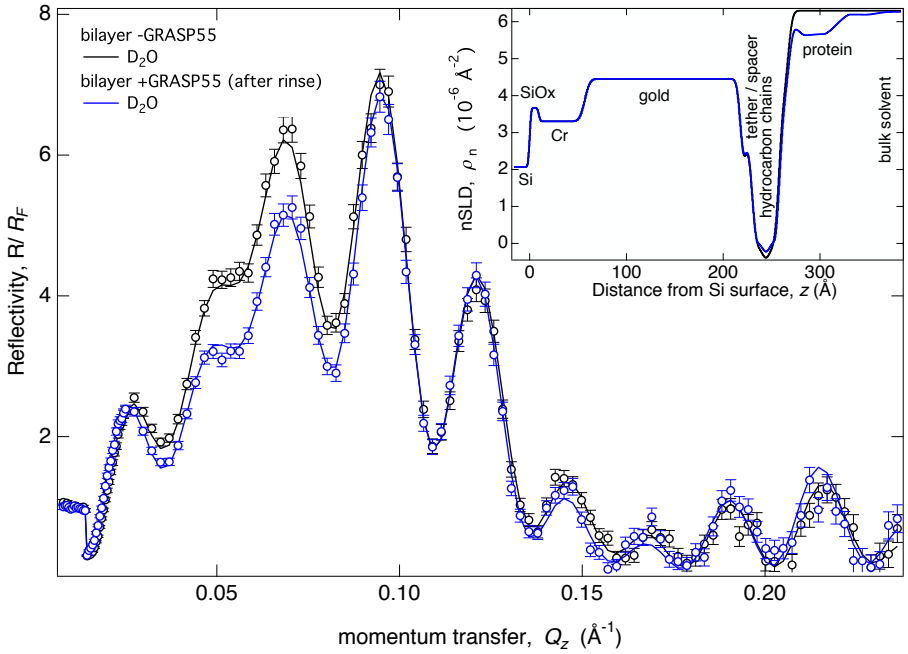
Data reduction using the NCNR 'reflpak' reflectometry package [Kie06]. The reflectivity data set was obtained from a similar system, a solid supported bilayer. The green curve shows the raw specular reflectivity for the sample in contact with a D_2O-based buffer. The black and blue curves are background radiation. In order to calculate the neutron reflectivity, the background intensity is subtracted from the specular reflectivity and the difference is divided by the incident beam intensity (not shown). This ratio, the neutron reflectivity, is always ≤ 1.

Data Analysis

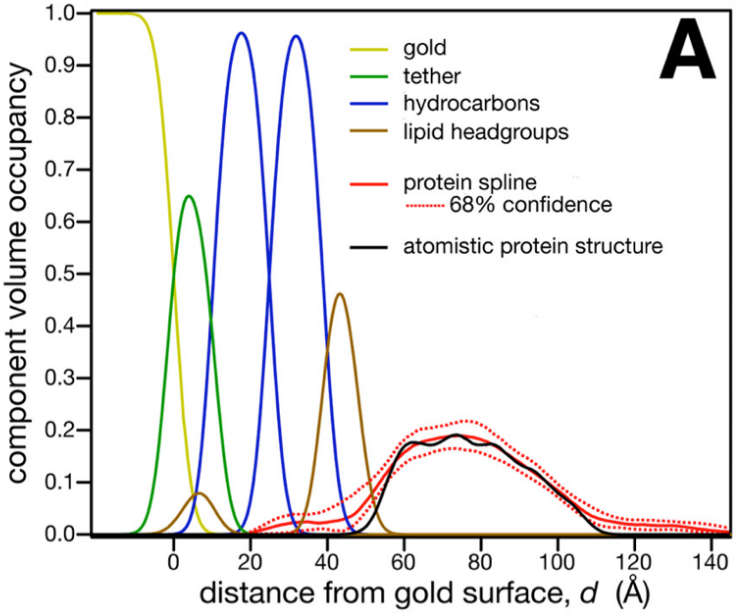
After reducing the neutron reflectivity data, the data is fitted to a model of the neutron scattering length density (nSLD) profile along the axis perpendicular to the substrate surface. In the simplest approach, the nSLD profile is modeled in terms of layers of constant nSLD. This type of model is called a 'box model'. Layer thicknesses, layer nSLD, and interlayer roughness are model parameters that are determined by the fit procedure. For data fitting using a box model, the NCNR reflectometry package 'reflpak' will be used [Kie06]. More advanced modeling techniques [Hei14] will be introduced.

Within a box model, the fitted nSLD profile is structurally interpreted in terms of chemically distinct layers. In the case of a tethered lipid bilayer, the following order of layers is used: the silicon substrate, the silicon oxide layer, the hydrated tether layer between the substrate and the bilayer, the inner headgroup layer, the hydrocarbon region of the bilayer,

the outer headgroup layer, and the bulk solvent phase. From the nSLD and thickness of the hydrocarbon layer the area per lipid molecule can be obtained. From simultaneous fits of the data sets with isotopically different bulk solvent phases, the hydration of the various layers and the completeness of the lipid bilayer can be calculated. Using advanced modeling techniques, those quantities can be directly parameterized and determined.



Neutron Reflectivity, fit, and nSLD profile for a system similar to the tethered lipid bilayer prepared during this tutorial experiment (only D_2O-based bulk solvent contrasts shown). The two data sets show the tethered lipid bilayer before and after incubation with protein (GRASP55 [Hei14b]).



Advanced modeling shown for the data set with membrane-associated GRASP55. Instead of homogeneous slabs of constant scattering length density (box model), volume distributions of molecular groups are modeled (composition-space model). Therefore, quantities of interest can be directly obtained from the fit. Here, for example the penetration of the GRASP protein into the lipid bilayer and the orientation of the protein using an atomistic protein structure was determined [Hei14b]).

References

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