Experimental Report

Proposal: **1-20-35** **Council:** 10/2012

Title: Direct Localization of Membrane-Bound Proteins with Grazing-IncidenceNeutron Fluorescence

This proposal is continuation of: 1-20-15

Researh Area: Methods and instrumentation

Main proposer: **SCHNECK EMANUEL**

Experimental Team: SCHNECK EMANUEL

Local Contact: DEME Bruno

Samples:
Gd-labelled streptavidin
SOPC (phospholipid)
DOPE-Cap-Biotinyl (phospholipid with biotin headgroup)
GdCl3
NaCl
HEPES buffer

Instrument	Req. Days	All. Days	From	To
D16	10	6	30/04/2013	07/05/2013

Abstract:

With the planned experiments we aim at the precise and unambiguous localization by of Grazing-Incidence Neutron Fluorescence (GINF) of proteins bound to solid-supported lipid membranes. The membranes contain lipids with biotinylated headgroups at defined concentrations to which Gd-labelled streptavidin proteins are specifically bound from the aqueous medium. With the experiments we will explore the sensitivity and spatial resolution with which biological interfaces composed of complex biomolecules can be probed by GINF.

EXPERIMENTAL REPORT

EXPERIMENT N° **1-20-35** INSTRUMENT D16

DATES OF EXPERIMENT 30/04/2013 to 07/05/2013

TITLE Direct Localization of Membrane-Bound Proteins with Grazing-IncidenceNeutron Fluorescence

EXPERIMENTAL TEAM
Emanuel Schneck (ILL), Bruno Demé (ILL), Michael Jentschel (ILL)

LOCAL CONTACT Bruno Demé

Date of report 14/01/2014

We have recently established Grazing-Incidence Neutron Fluorescence (GINF, see Fig. 1 left) as a tool for the detection and localization of labeled molecules near the solid/liquid interface using an evanescent neutron wave [1]. In analogy to Grazing-Incidence X-ray Fluorescence (GIXF) [2-4] applicable at the air/liquid interface, GINF at the solid/liquid interface has great potential in providing molecule-specific structural information to complement x-ray and neutron scattering studies in the fields of soft-matter and biology, such as studies of solid-supported cell-surface models [5].

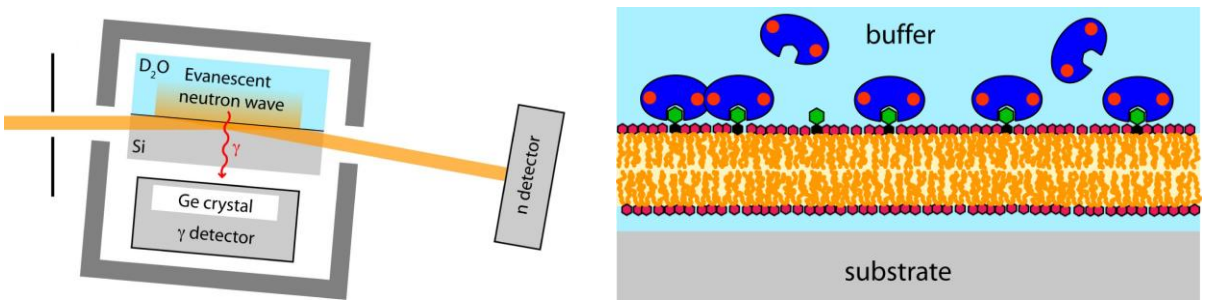

Figure 1: (left) Schematic illustration of a GINF experiment for the localization of labeled molecules at the solid/liquid interface. (right) ^{157}Gd-labeled proteins bound to the surface of a solid-supported lipid membrane.

The aim of Experiment 1-20-35 was to directly localize ^{157}Gd-labeled proteins bound to a membrane surface using GINF at the solid/liquid interface (Fig. 1 right). We used lipids with biotinylated headgroups at defined concentrations to which labeled streptavidin proteins bind specifically.

In the first step the architecture of the protein-decorated supported membrane was determined by monochromatic reflectometry (λ = 0.472 nm and $\Delta\lambda/\lambda$ = 1%). The reflectivity curve and the corresponding scattering length density slab model, displaying silicon substrate, oxide and water layers, membrane, and proteins, are shown in Fig. 2 (left and middle). The sample was then illuminated at small incident angles around the critical angle of total reflection, in order to establish an evansecent neutron wave near the interface. Via the characteristic neutron capture γ signal of ^{157}Gd at 182 keV we could clearliy detect the ^{157}Gd-labeled streptavidin at the solid/liquid interface. By changing the incident angle we systematically modified the shape of the standing neutron wave at the solid/liquid interface. The resulting angle-dependent fluorescence intensity is consistent with a model representing the label distribution as a broad Gaussian distribution coinciding with the protein layer as determined by reflectometry. The statistics of the fluorescence signal are, however, too poor for an exact characterization of the label distribution. Since the protein layer is clearly visible in the reflectivity measurement, we attribute the poor statistics to un-effective protein labeling.

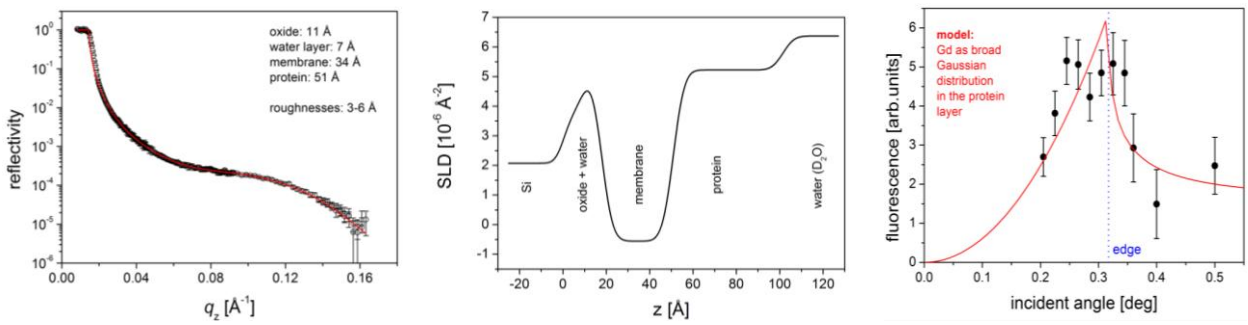

Figure 2: (left and middle) Reflectivity curve and slab model of the scattering length density (SLD) profile of the protein-decorated supported membrane. (right) Normalized fluorescence intensity from the protein layer as a function of the incident angle. The line is a theoretical predictions for a broad label distribution coinciding with the protein layer as determined by reflectometry.

References

[1] Schneck, Jentschel, Gege, Tanaka, Demé, *Langmuir* **29**, 4084 (2013)

[2] Yun & Bloch, *J. Appl. Phys.* **68**, 1421 (1990).

[3] Padmanabhan et al., *Physical Review Letters* **99**, 086105 (2007)

[4] Schneck et al. *Proc. Natl. Acad. Sci. USA* **107**, 9147 (2010)

[5] Tanaka & Sackmann, *Nature* **437**, 656 (2005)

Edition May 2000