Experimental report

14/09/2023

Proposal: CRG-2965 **Council:** 10/2022

Title: The use of magnetic reference layers to unravel interaction of proteins with complex lipid bilayers

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Tommy NYLANDER

Experimental team: Tommy NYLANDER
Marshall MACHINGAUTA
Alessandra LUCHINI

Local contacts: Anton DEVISHVILI

Samples: Myoglobin
Monoolein
Diglycerolmonooleate (DGMO)
Polysorbate 80

Instrument	Requested days	Allocated days	From	To
SUPERADAM	5	3	01/06/2023	04/06/2023

Abstract:

In previous work, we have characterised the encapsulation of several industrially relevant proteins, aspartic protease, ß-galactosidase) and sugar beet phytoglobin into lipid sponge phases. In the present study we will investigate important aspects of the encapsulation of iron binding hem-proteins, e.g. myoglobin, in the sponge phase, namely the protein lipid interaction. These type heme-bound iron are used to treat anemia instead of iron in organic salts conventionally used. Plant haemoglobins (or phytoglobins) are here specially interesting as the address one of the challenges with plant-based food, namely supply of sufficient iron that has high bioavailability. The reason for encapsulation is preventing unwanted proteolytic reaction and redox reactions. Neutron reflectometry is the only technique that can be used to characterise the interaction between the heme protein and the lipid at the interface. Here we will utilise the difference in scattering length density between a protein and hydrogenated lipid in combination with solvent contrast matching. The use of magnetic reference layers provides additional contrasts.

Experimental report (proposal CRG-2965)

Scientific Background. This experiment is part of a larger project focused on the incorporation of proteins such as Aspartic protease or myoglobin, which are common food ingredient in e.g. cheese manufacturing, in lipid particles composed predominantly of phospholipids, i.e. DOPC and DOTAP, glyceryl monooleate (GMO) and diglyceryl monooleate (DGMO). The lipid formulation could offer large protection and the use of food grade ingredients, but also give the opportunity to more precisely regulate the activity by slow release from the lipid phase. These lipid formulations can produce bilayer-like structures on hydrophilic surfaces such as silicon oxide. Therefore, neutron reflectivity (NR) is a suitable technique to both characterize the structure of the bilayer structure as well as to investigate the protein incorporation. In this experiment on SuperADAM we used standard monocrystalline silicon substrates with silicon substrates including a magnetic reference layer composed of iron. Such substrate has a thick (~800 Å) SiO2 capping layer, which protects the magnetic iron layer from chemical degradation (e.g. if exposed to saline solution) and at the same time provides the same silica interface as standard silicon crystals commonly used for NR experiments. The use of this magnetic substrates is particularly important to avoid modification of the lipid bilayer structure because of the solvent rinsing step normally needed for contrast variation. Indeed, by performing polarized neutron reflectometry experiments, we exploited the different interaction with the magnetic substrates of spin up and spin down neutrons to achieve 2 different contrasts with respect to the sample.

Experimental data. Figure 1a shows the experimental data collected for one of the 3 substrates with magnetic reference layer that were used for this experiment. All substrate exhibited a comparable structure including: i) approx.. 43 Å silicon oxide layer on top of the silicon substrate; ii) approx. 100 Å iron layer; and iii) approx. 1000Å silicon oxide layer as outer most layer. This result is in good agreement with previous characterisation of this kind of substrate. Figure 1a also shows that the reflectivity data collected with neutron spin polarisation up and down exhibit a considerably different trend, as a consequence of the interaction with the magnetised iron layer. The magnetic field used for the experiment was 0.01T. Data for one of the substrates was collected also in different solvents, i.e. D2O, 38%D2O 62%H2O and H2O. Simultaneous fit of the data in different contrast as compared to the analysis only for the D2O contrast, produced similar results for the substrate structure.

Figure 1b shows the data collected after the injection of the lipid formulation. In this experiment we tested three different lipid formulations: i) 70%[DGMO(10):DOPC(30):GMO(60)]+30% P80; ii) 70%[99%(DGMO(10):DOPC(30):GMO(60))1%DOTAP]+30% P80 and iii) 70%[DOPC(40):GMO(60)]+30% P80. Figure 1b corresponds to formulation iii. In all cases we observed the formation of a supported lipid bilayer with good surface coverage (≥85%). The structure of the bilayer was modelled as a stack of three layers, two of them representing the inner and outer polar headgroups and an intermediate one representing the hydrophobic acyl chains. In all three cases, the bilayer structure was similar with headgroup thickness of 7-12Å and scattering length density of $1.8 \cdot 10^{-6} Å^{-2}$, and acyl chain thickness of 27-31 Å and scattering length density of $-0.3 \cdot 10^{-6} Å^{-2}$. These results were obtained using only data collected in d-buffer

(20mM TRIS pH=7) and by simultaneously analysing the data corresponding to spin polarization up and down.

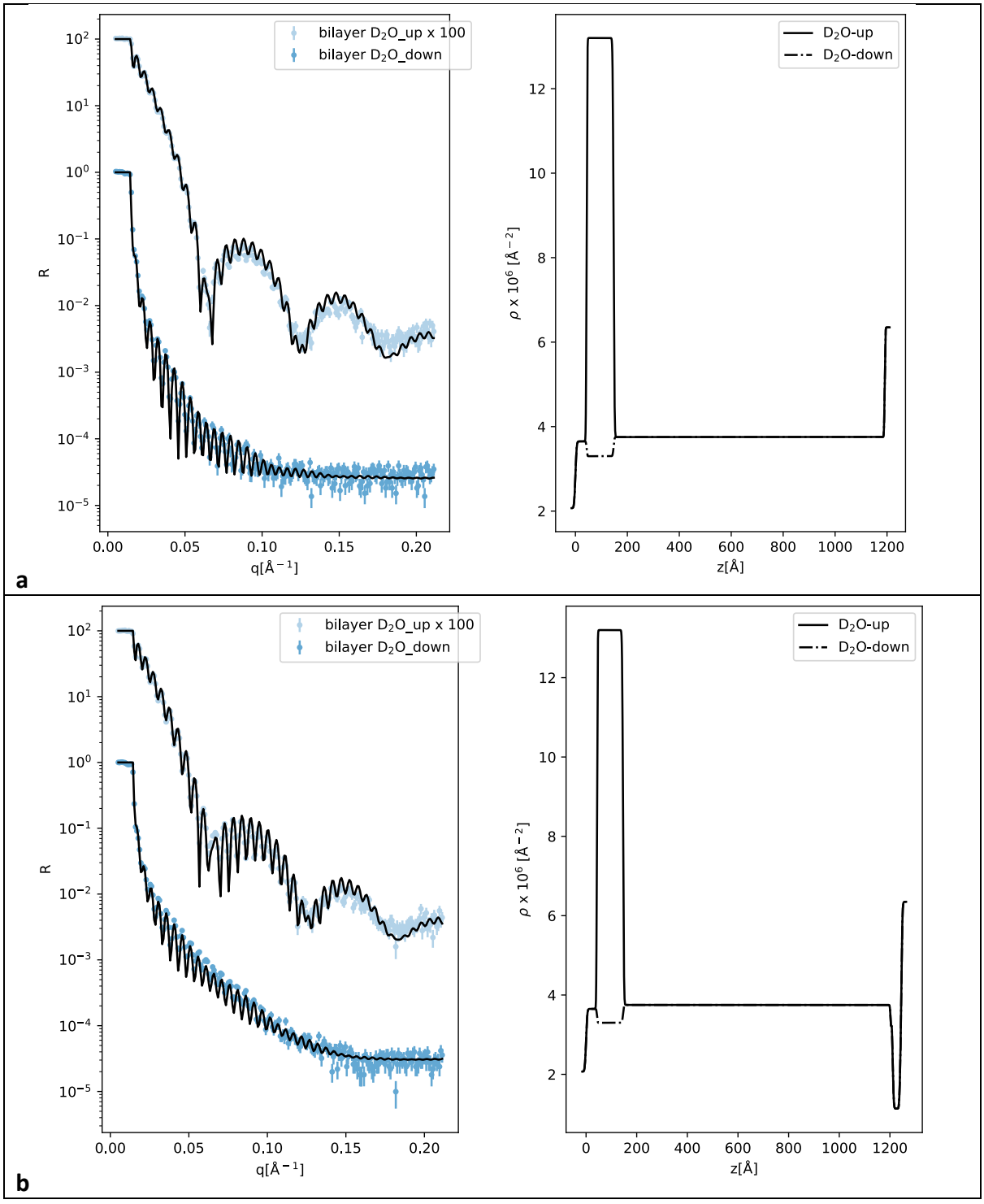

Figure 1: NR data together with the corresponding fitting curve and scattering length density profile for the bare substrate a) and after the injection of the lipid formulation b).

Conclusions. Altogether we demonstrated the use of substrate with a magnetic reference layer and outer silicon oxide capping layer as suitable for the characterisation of lipid bilayers formed by formulations of relevance for food science. In a follow-up experiment we will test the incorporation of proteins such as Myoglobin in the lipid bilayer.