

Experimental report

15/02/2020

Proposal: 8-02-801

Council: 4/2017

Title: Omega-3 fatty lipids as modulating agents of the biomembranes microstructural properties: the role in cholesterol positioning

Research area: Soft condensed matter

This proposal is a continuation of 8-02-703

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Samples: Cholesterol (C27H46O)
POPC (C44H84NO8P)
22:6(cis)PC (1,2-docohexanoyl- sn-glycero-3-phosphocholine)

Instrument	Requested days	Allocated days	From	To
FIGARO	3	0		
D17	3	2	18/06/2018	20/06/2018

Abstract:

Polyunsaturated omega-3 fatty acids are increasingly proposed as dietary supplements because they have been associated with a strong neuroprotective effect. We would like to investigate by Neutron Reflectometry (NR) the role played by 22:6(cis)PC (1,2-docohexanoyl-sn-glycero-3-phosphocholine) fatty lipid, which is a di-polyunsaturated acyl chain phospholipid present in the mammalian brain, on the microstructural properties of biomimicking membranes formed by 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (POPC) and POPC/Cholesterol. Our interest is focused on the different behaviour of phospholipid containing one or two polyunsaturated omega-3 acyl chains and on the role in modulating the cholesterol positioning in the bilayers by using deuterated lipids. On the basis of the obtained information, the role of omega-3 lipid on the interaction between biomembranes and amyloidogenic peptides will be also studied. The investigation will be realized by using the method of solvent contrast variation and deuterated phospholipids.

Structural characterization of supported membrane hosting Tissue Factor

Introduction Recently, we are investigating the formation of supported membranes by depositing on a solid substrate protein-loaded nanodiscs (ND). Nanodiscs are discoidal lipid bilayers surrounded by a protein belt. They are ~ 10 nm particles initially introduced by the Sligar group [1] and successfully exploited to reconstitute several membrane proteins and investigate their structure in solution [2]. The present work is based on a “cut-up” version of the NDs, named peptide-discs, where the nanodisc belt is composed by self-assembled peptide (named 18A) molecules [3]. The peptide-discs are characterized by a very dynamic structure so that it is possible to remove the peptide belt from the formed lipid bilayer by washing the deposited NDs with buffer. In this NR experiment, we proposed to use the peptide disc deposition to produce and subsequently characterize supported lipid bilayers containing Tissue Factor (TF) molecules. TF is the membrane protein that initiates blood coagulation upon binding with the soluble protein activated factor VII (FVIIa). Anionic phospholipids, specifically phosphatidylserine (PS), are suggested to trigger TF-activation towards blood coagulation by interacting both with TF and FVIIa [4]. Atomic structures are available for the TF extracellular domain in complex with FVIIa [5]. However, there is no structural information available on the full-length and membrane-anchored TF or on the TF/FVIIa complexes in lipid membranes, where they play their essential biological role. Hence, we are interested in the characterization by neutron reflectometry of TF and TF/FVIIa structure in supported lipid bilayers prepared with either the zwitterionic phospholipid (1-palmitoyl-2-oleyl-3-*sn*-glycerophosphatidylcholine) or a mixture of POPC and the anionic phospholipids POPS (1-palmitoyl-2-oleyl-3-*sn*-glycerophosphatidylserine).

Experimental Results. Figure 1 shows the NR experimental data collected for the POPC and POPC/POPS supported lipid bilayers with TF. The very low signal in the deuterated buffer suggests a low surface coverage ($\sim 50\%$ as estimated from a preliminary data fitting of data collected in three different contrasts). This result was really unexpected since supported lipid bilayers with TF were repeatedly

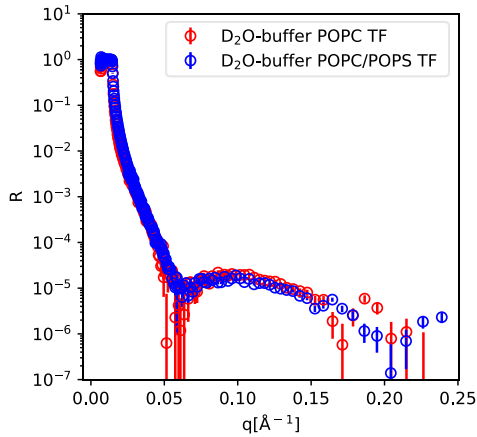


Figure 1: NR experimental data collected for POPC supported lipid bilayer with TF (red) and POPC/POPS supported lipid bilayer with TF (blue).

produced with high surface coverage ($\geq 80\%$ v/v) during previous neutron reflectometry experiments as reported in a recent publication [6] and according to the data included in a manuscript in preparation. Further attempts to prepare the supported lipid bilayers with TF during this NR experiment produced similar results. This suggests that the observed low coverage was not related to a not 100% hydrophilic substrate surface, but the TF peptide disc solutions that were injected in the NR cell. We have evidences that in these samples TF was not properly reconstituted in the peptide-discs and that it might have formed aggregates in solution which could not be efficiently separated from the TF-loaded peptide disc during sample preparation. Because we have previously and successfully used the TF-loaded peptide discs to form supported lipid bilayers with TF molecules, we are confident that our sample preparation method works and that the low coverage observed during this NR experiment is only related to an unfortunate sample batch, where the TF was probably misfolded and aggregated. This has never occurred during our previous NR experiments and was therefore hard to predict. Now that we are aware of this potential issue during sample preparation, we will combine different techniques, i.e. size-exclusion chromatography (to purify the TF-loaded peptide discs), light scattering (to check the presence of large aggregates) and SDS-page to assess the protein aggregation state, to fully assess the quality of the TF-loaded peptide disc solution which will be use during future NR experiments.

Although we could not fulfil the structural investigations of TF in supported lipid bilayers of different lipid composition, we still made a successful use of the beamtime by testing new samples that we wish to further investigate in the future. In particular, we implemented the peptide disc deposition to produce supported lipid bilayers with a mixture of phospholipids extracted from yeast cells. These test measurements are part of a collaboration with the PSCM lab at ILL, where natural hydrogenous and deuterated phospholipids (hPol and dPol) are extracted from *Pichia Pastoris* yeast cells grown in hydrogenous and deuterated culture media [7, 8]. These phospholipids mixtures have a great relevance in the context of producing biological membrane models.

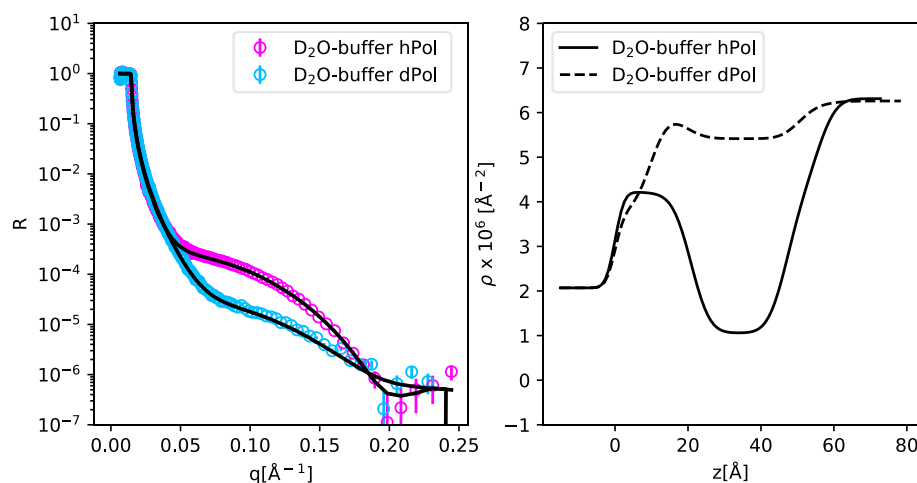


Figure 2: NR data collected in the buffer prepared with 100% D₂O for the hPol and dPol supported lipid bilayers prepared through peptide disc deposition.

Figure 2 shows the NR data collected in the buffer prepared with 100% D₂O for the hPol and dPol supported lipid bilayer. NR data were collected in three different contrasts (d-buffer=100% D₂O; smw-buffer=38% D₂O; 82% H₂O (for the hydrogenous lipids), 4mw-buffer=66% D₂O; 34% H₂O (for the deuterated lipids) and

h-buffer= 100% H₂O) and the fits reported in Figure 2 are the results of the simultaneous analysis of all the collected data. Overall, the deposition of peptide discs resulted to be a successful method for producing supported lipid bilayer composed by natural phospholipids and with a high surface coverage ($\geq 80\%$ v/v). These measurements allowed us to verify that our supported lipid bilayers preparation protocol is perfectly compatible with the yeast phospholipid extract.

Conclusions. In this experiment, we aimed at characterizing TF in supported lipid bilayer of different lipid composition. Unfortunately, due to a misfolding of TF in the peptide discs the preparation of the supported lipid bilayers was compromised and we were only able to obtain samples with a very low surface coverage. This phenomenon was not observed during previous neutron reflectometry experiment where we successfully produced and characterized POPC/POPS supported lipid bilayers incorporating TF molecules and was therefore very unexpected. Now that we are aware of this potential issue, a more detailed characterization of the TF-loaded peptide disc solutions will be performed in advance to evaluate the quality of the peptide disc solutions, which will be used during future neutron reflectometry experiments.

During this NR experiments we also performed some test measurements for the formation of supported lipid bilayers composed by natural phospholipids through peptide disc deposition. The collected data shows that supported lipid bilayers with either hydrogenous and deuterated phospholipids were successfully obtained. These test measurements will allow us to design future experiments in which we plan to characterize membrane proteins in supported lipid bilayers composed by these natural phospholipids as a more complex and realistic model of biological membranes.

References:

- [1] T. H. Bayburt *et al.*, Nano Letters, 2002, 2, 853-856.; [2] N. Skar-Gislinge *et al.*, Acta Crystallographica Section D, 2015, 71, 2412-2421; [3] S. R. Midtgaard, Soft Matter, 2014, 10, 738-735; [4] A. Ansari *et al.*, PloS One, 2016, 11, 1-16; [5] D. W. Banner *et al.*, Nature, 1996, 380, 41-46; [6] A. Luchini *et al.*, Analytical Chemistry 2020, 92, 1, 1081-1088. [7] A. Luchini *et al.* Colloids and Surfaces B; 2018,168,126-133; [8] A. Luchini *et al.* Chemistry and Physics of Lipids; 2020, 227, 104873;