Experimental report

15/02/2020

Proposal: 8-02-874 **Council:** 4/2019

Title: Structural characterization of supported membrane hosting Tissue Factor

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: POPS
POPC
Tissue Factor
factor VIIa
18A

Instrument	Requested days	Allocated days	From	To
D17	3	3	04/02/2020	07/02/2020
FIGARO	3	0		

Abstract:

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. The present work will be based on a on a “cut-up” version of the NDs, named peptide-discs, where the nanodisc belt is composed by self-assembled peptide (named 18A) molecules. Recently, we have so far conducted a pilot study which show that the formation of a macroscopic lipid bilayer can indeed be mediated by peptide-disc deposition and subsequent removal of the peptide molecules by buffer rinsing. We are now interested in testing the method for the preparation of supported membranes hosting oriented membrane proteins. The membrane protein we propose to investigate is Tissue Factor (TF). TF is the membrane protein that initiates blood coagulation upon binding with the soluble protein activated factor VII (FVIIa). Here we propose to produce supported membranes hosting TF through deposition of TF-loaded peptide-discs and to characterize their structure by means of NR.

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 be based on a 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 (~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 produced with high surface coverage (≥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.

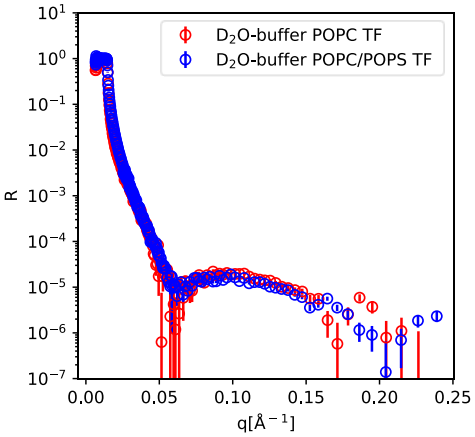

Figure 1: NR experimental data collected for POPC supported lipid bilayer with TF (red) and POPC/POPS supported lipid bilayer with TF (blue).

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.

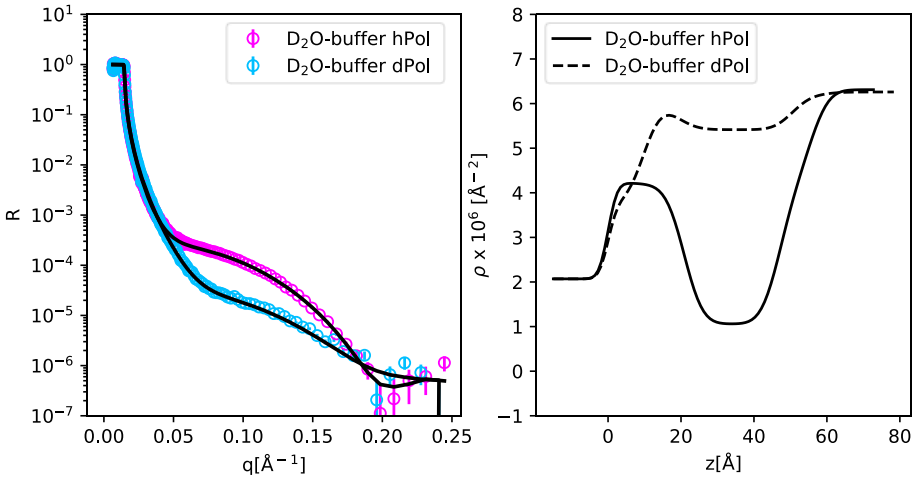

Figure 2: NR data collected in the buffer prepared with 100% D2O for the hPol and dPol supported lipid bilayers prepared through peptide disc deposition.

Figure 2 shows the NR data collected in the buffer prepared with 100% D_2O for the hPol and dPol supported lipid bilayer. NR data were collected in three different contrasts (d-buffer=100% D_2O; smw-buffer=38% D_2O 82% H_2O (for the hydrogenous lipids), 4mw-buffer=66% D_2O 34% H_2O (for the deuterated lipids) and h-buffer= 100% H_2O) 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 (≥ 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;