Experimental report

17/09/2018

Proposal: 9-13-731 **Council:** 4/2017

Title: Interaction of cellulose nanocrystals with lipid membranes

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Lipids
Cellulose

Instrument	Requested days	Allocated days	From	To
D17	5	0		
FIGARO	5	3	03/04/2018	06/04/2018

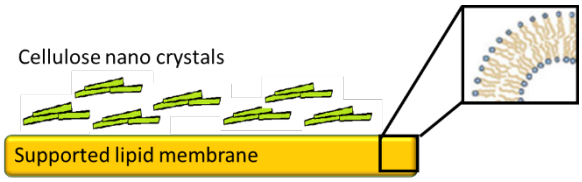
Abstract:

A better understanding of the structure of plant cell walls is a major challenge from both soft matter and biological points of view. In the plant kingdom, one of the first stages of plant cell wall construction is the deposition of cellulose microfibrils on the external leaflet of a lipidic membrane. In this context, CERMAV has recently developed biomimetic constructs in which cellulose nanocrystals (CNCs) were deposited on the surface of a lipid membrane, serving both as a basic model of plant cell wall and as innovative biomaterials. Preliminary QCM-D experiments allowed us to identify some key parameters governing this interaction. However, the structural features of such 2D constructs are yet to be investigated. In the proposed experiment, neutron reflectivity will be used to study the influence of pH, CNC concentration and the type of lipids (including the use of biologically relevant glycolipids) on the dimensions, composition and morphology of the formed assemblies, which will lay the foundation for the creation of novel biomimetic constructs made from CNCs and lipids.

Cermav / ILL NEUTRONS FOR SCIENCE	Experiment *9-13-731 FIGARO; 3-6 th April, 2018* Local contact: Giovanna Fragnieto Experiment Report	12.09.18 Report by: Yotam Navon PI: Laurent Heux Bruno Jean Sebastien Mongrand

1. *Background and objective*

Experiment 9-13-731 was carried out in the framework of the Ph-D thesis of Yotam Navon, which aims at designing biomimetic materials inspired from the Plant Cell Wall (PCW). Specifically, the experiment aimed at investigating the interaction of lipid membranes with cellulose. The plant plasma membrane contains several lipid families, and in order to better mimic the natural composition, it is crucial to incorporate lipids from each representative group (i.e. phospholipids, glycolipids and sterols) in the models used for this purpose. An important question to address is the ability to form biomimetic lipid bilayers that contains up to 30 % mol. glycosyl inositol phosphoryl ceramides (GIPC), a major glycolipid. Cellulose nanocrystals (CNCs) from cotton were chosen to mimic the cellulose fibers, present naturally in the primary cell wall of growing plants. Experiment 9-13-731 was therefore designed to:

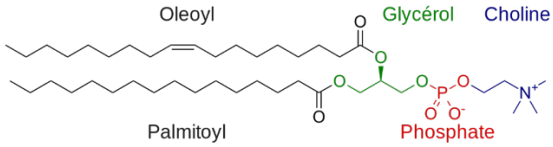
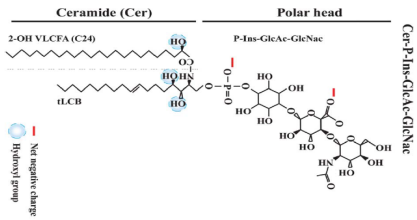
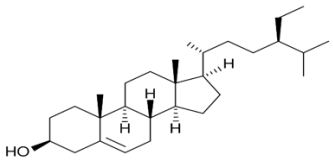


a. Investigate the formation of lipid membranes from several compositions.
b. Investigate the architecture of the multilayer films following the deposition of CNCs.

2. *Experimental setup*

We have tested three different lipid compositions, which were deposited onto a silicon substrate by small unilamellar vesicles (SUVs) fusion. Each lipid represented a structural group present in the plasma membrane. POPC (phospholipids), GIPC (Glycolipids) and Sitosterol (Sterols) were mixed in such a way that the total lipid concentration was 1 g L-1 and the molar fraction of GIPC varied between experiments. Finally, CNCs were deposited on the lipid layer (Figure 1). The reflectivity was measured for the bare silicon substrate and after each deposition step with 4 different solvents: D2O, H2O, silicon-matched water (SMW, SLD = 4.10-6 Å-2) and 4-matched water (4MW, SLD = 4.10-6 Å-2). The experiments are denoted P1 P2 and P3 for 0, 15 and 30 %mol GIPC respectively. Table 1 shows the chemical structure and molar ratio of the lipids used.

Table 1-The structure and molar ratio of the lipids and the lipid mixtures.

Lipid	Chemical structure	Molar ratio		
		P1	P2	P3
POPC		70	55	33
GIPC		-	15	33
B-Sitosterol		30	30	33

3. *Results*

3.1. SLB formation

Figure 2 shows the reflectivity spectra and the corresponding calculated SLD profiles, following the SLB deposition. The multilayer model was composed of the silicon substrate (SLD=2.07 10-6Å-2) covered with a layer of silicon oxide (SLD=3.47 10-6 Å-2). No additional water layer was introduced in the model between the substrate and the lipid polar head group. For the lipid head group, in P1 we used the SLD of POPC (1.8 10-6 Å-2) whereas in P2 and P3, that contain GIPC, an additional layer of sugars was assumed, with SLD of 1.9 10-6 Å-2. The hydrophobic region was represented by a single layer with SLD of -0.31 10-6 Å-2. The fitting procedure was done using Motofit and Aurore softwares.

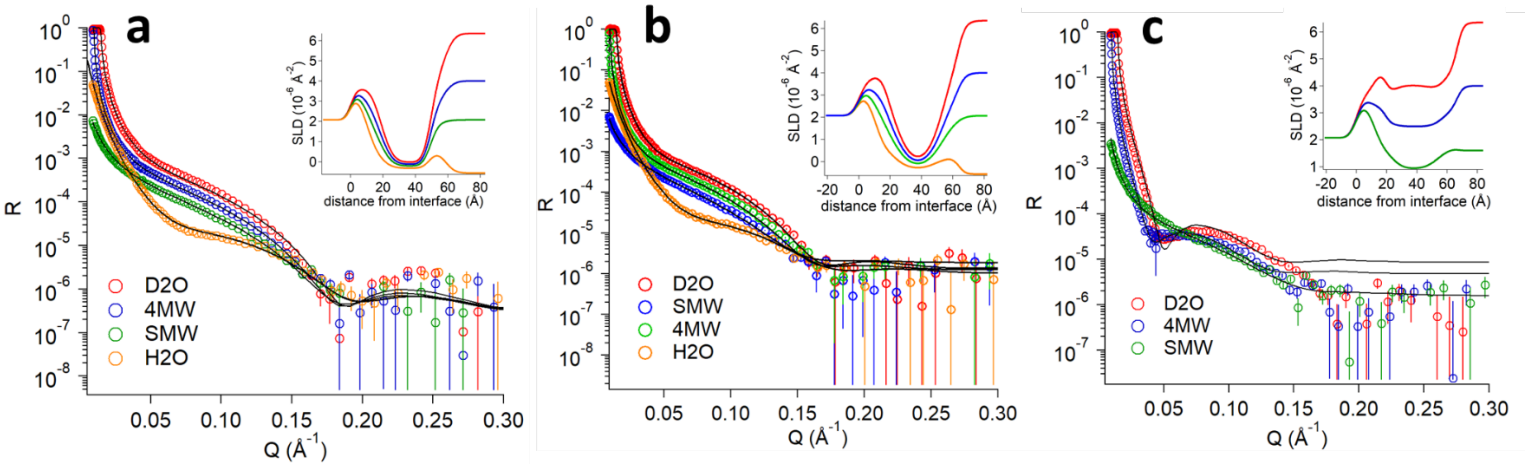


Figure 1- Reflectivity and SLD (inset) profiles of SLBs on silicon substrate. P1 (a) P2 (b) and P3 (c) (see Table 1).

As can be seen from the plots in Figure 1, a good fit was obtained with a single set of parameters for all four solvents for P1 and P2 (containing 0 and 15% mol. GIPC respectively). However, for P3 with 33% mol. GIPC, we could not obtain a good fit for any of the solvents. Moreover, the closest fit yielded a solvent content in the lipid hydrophobic region of ~65%, a value which is significantly higher than the one expected for a bilayer with full coverage. It is possible that the presence of high amount of glycolipid altered the membrane properties in such a way that the deposition protocol used was no longer efficient in inducing bilayer formation. The parameters obtained from the fitting of the reflectivity profiles are summarized in Table 2:

Table 2. Structural parameters of the SLBs obtained from the fitting of the reflectivity data.

Parameter	P1	P2	P3
Bilayer formation	Yes	Yes	No
% solvent in the tails	5	5	65
Inner Sugar[Å]	-	4	4
Inner Head[Å]	8	8	8
Tail[Å]	31	31	31
Outer Head [Å]	8	8	7
Outer Sugar [Å]	-	4	

Table 3. CNC layer properties after adsorption on P2 and P3 the fitting of the reflectivity data.

Parameter	P1	P2
CNCs thickness [Å]	77	75
%solvent in CNCs	70	85
Roughness [Å]	25	25

The thickness values obtained for P1 (POPC/Sitosterol 70/30 % mol.) were 31 Å for the tails region, and 8 Å for both the inner and outer hydrophilic head. The solvent content was 5 % for the tails and about 50 % in the head groups. The thickness values obtained for P2 were 31 Å for the tails region, 4 Å for both the inner and outer hydrophilic head and 8 Å for both the inner and outer sugar head respectively. The solvent content was 5 % for the tail region, a reasonable result for a bilayer. The solvent content was about 50 % for the phospholipid layer and 80 % for the sugar head layer. The high solvent content in the sugar heads may be explained by the low content of GIPC int the membrane (15 % mol.) however, for the phospholipid part, the amount of solvent was high compared to previous observations on POPC membranes (Montis, 2016). As mentioned above, for P3 we could not obtain a proper fit, implying that a bilayer was not formed in this case.

3.2. CNC deposition

Figure 3 shows reflectivity spectra and SLD profiles for P1 and P2 following the deposition of CNCs on top of the bilayer. Data were fitted using the parameters obtained for the SLB with an additional CNC layer. No attempt was done to deposit CNCs on P3, as the bilayer was not formed properly. In order to mediate the adsorption of CNCs on the negatively charged membrane, a rinsing step with 5 mM Ca2+ solution was performed. The thickness obtained for the CNC layer was 7.7 and 7.5 nm for P1 and P2 respectively. The roughness was 25 Å and the amount of solvent was 70 and 85 % for P1 and P2 respectively.

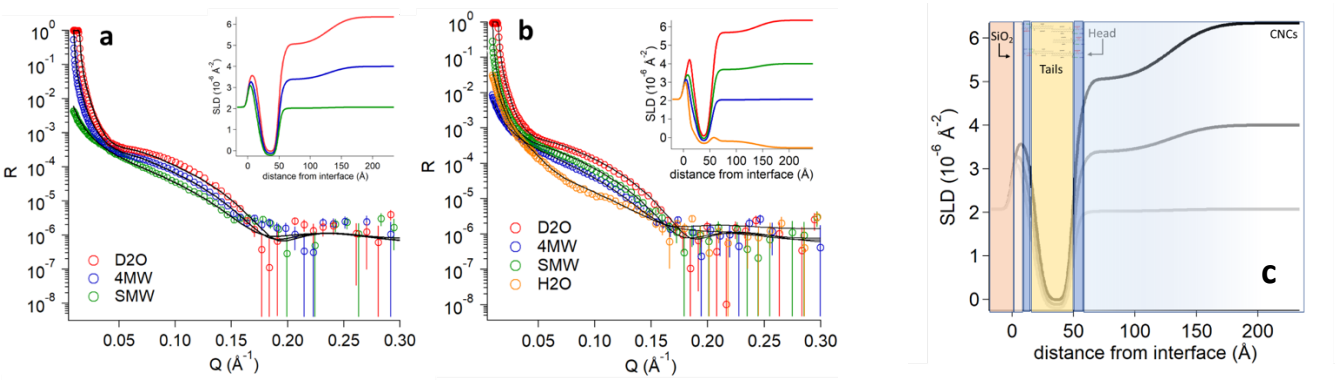


Figure 2- Reflectivity spectra and SLD profiles (inset) of P1 (a) and P2 (b) following CNC deposition. Overlay of the multilayer model and SLD profile for P1 after deposition of CNCs

CNCs are rod-like objects with an approximately rectangular cross section, the smallest dimension of which is ~7-8 nm (height). The dimensions of the CNC layer are therefore in line with the values known from other observations for CNCs. However, the high content of solvent indicates that the CNC layer may not be continuous in contrast to what was observed by our group using QCM-D and AFM.

4. Summary and outlook

The structure and composition of SLB and SLB+CNC layers were investigated at FIGARO. For the lipid bilayer deposition, good fits were obtained for low content GIPC membranes (P1 and P2) suggesting the formation of a complete bilayer. However, in experiment P3 (33% mol GIPC) a bilayer was not formed as indicated by the high solvent content in the hydrophobic tails region. For the deposition of CNCs, for both P1 and P2, the model yields a layer with SLD of 1.9 Å^{-2}, thickness of about 7 nm, high solvent content 70-85 % and roughness of 25 Å. The dimensions are reasonable considering the type of CNC used. Two additional issues that require further investigation, appears from the results:

1) The deposition of high GIPC content SLBs was not possible, it is therefore crucial to develop a deposition protocol that will enable their formation. Bearing in mind we were able to obtain a bilayer with 15 % mol. GIPC (P2) but not with 30 % Mol. (P3), a possible route will thus be the preparation of a series of samples with increasing GIPC content (15, 20, 25 and 30% mol.). This will help to obtain a quantitative threshold for GIPC incorporation in synthetic SLBs. It is worth mentioning that in a preliminary study using QCMD, high content GIPC liposomes were used to induce a complete bilayer formation. However, the surface area and the roughness of the substrate differed, as well as the substrate surface pre-treatment. In order to avoid time loss in future beam-time, a preliminary study may be performed using similar substrate investigated by grazing incident X-ray scattering, which will enable the optimization of the protocol prior to the experiment.

1. An improved deposition protocol for the CNC layer needs to be developed. It is expected that CNCs will create a denser layer with lower amount of solvent, as observed in AFM and QCM-D measurements for other types of lipid compositions. For example, the presence of positively charged lipids such as 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) in the membrane composition up to 10% mol., may induce electrostatic attraction and enhance CNC deposition as seen previously by our group on lipid vesicles and SLBs.