

Experimental report

24/01/2024

Proposal: 9-13-1091

Council: 4/2023

Title: Mimicking the interface between mammalian plasma membrane and extracellular matrix: chondroitin sulphate-decorated supported lipid bilayers

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Cs
POPC, POPS, CHOL

Instrument	Requested days	Allocated days	From	To
FIGARO	4	4	06/12/2023	10/12/2023
D17	4	0		

Abstract:

Several vital processes, such as signal transduction and interaction with pathogens or drugs, take place on the cell surface, and specifically at the interface between the plasma membrane (PM) and the extracellular matrix (ECM). The phospholipids such as the zwitterionic phosphatidylcholine (PC), the negatively charged phosphatidylserine (PS) and cholesterol (CHOL) are among the most abundant lipids in the PM. The ECM is an additional layer bound to the PM, which is composed of flexible carbohydrates, like chondroitin sulphate CS. CSs are over-expressed in abnormal cells such as cancer cells. We propose to perform NR measurement to determine the scattering length density profile of the CS molecules at a PC/PS/CHOL bilayer surface, from which the structure of the CS layer as function of the lipid composition can be obtained. Data will be collected at physiological pH and acidic pH to mimic the environment of cancer cells. This information is crucial to elucidate the role of the PM-ECM interplay in signalling processes both in normal and cancer cells. CS-SLBs will also represent more advanced cell surface models of great interest for interaction studies with proteins and drugs.

Mimicking the interface between mammalian plasma membrane and extracellular matrix: chondroitin sulphate-decorated supported lipid bilayers

Scientific Background. Several vital processes, such as the interaction with pathogens or drugs, take place on the cell surface, and at the interface between the plasma membrane (PM) and the extracellular matrix (ECM). The phospholipids such as the zwitterionic phosphatidylcholine (PC), the negatively charged phosphatidylserine (PS) are among the most abundant lipids in the PM [1]. The ECM is an additional layer bound to the PM, which is composed of flexible carbohydrates and proteins and is responsible for the cell organisation within tissues. Chondroitin sulphates (CSs) are widely present in the ECM of animal cells and are composed of a disaccharide unit (i.e. glucuronic acid and galactosamine), which can be sulfonated at different positions. Mono-sulfonation at position 4 or 6 is the most common, resulting in CS-A and CS-C species, respectively. Typically, CS-A is the most abundant form in human cells, however CS-C is overexpressed in abnormal cells such as cancer cells [2]. A previous study reported a protocol for the functionalisation of supported lipid bilayers (SLBs) with CS (named here CS-SLBs) [3]. Briefly, a modified phospholipid that bears an amino group exposed to the bulk solvent (18:1 Dodecylamine PE, DOPE-NH₂) is added to the SLB to form an amide bond with CS. CS-SLBs were mostly characterised with spectroscopy techniques and by using pure zwitterionic PC lipids or the negatively charged phosphatidylglycerol (PG) lipid for the SLB. The performed experiment on FIGARO was designed to pioneer the structural investigation of CS-functionalised SLBs. This is part of a larger project aimed at developing systems based on SLBs to mimic the interface, where an ILL-funded PhD student is involved.

Results. Prior to the NR experiment we collected QCM-D data to test the SLB functionalisation with CS. The collected data were consistent with those previously reported [3] and indicated an increase of the absorbed mass upon injection on the SLBs (including DOPE-NH₂) of a solution of CS together with NHS and EDC as activating agents at pH=5 (MES buffer). We also collected QCM-D data for the injection of CS on the same SLBs without NHS and EDC and observed no variation in the absorbed mass. This result supported the formation of an amide bond between CS and DOPE-NH₂. During the beamtime on FIGARO we adopted the same protocol as tested with QCM-D. We successfully formed by vesicle fusion SLBs with composition i) POPC/DOPE-NH₂ (70:30); ii) POPC/DOPE-NH₂ (50:50); iii) POPC/POPS/DOPE-NH₂ (50:20:30); iv) POPC/POPS/DOPE-NH₂ (30:20:50). Figure 1a, b shows the data collected for POPC/DOPE-NH₂ (50:50) together with the corresponding fitting curve. The data analysis indicated the formation of an SLB with high surface coverage (~99%). Similar results were obtained also for the other lipid compositions. This is quite an important result given the different structure of the DOPE-NH₂ as compared to the other phospholipids, i.e. POPC and POPS, for which vesicle fusion was already reported as a reliable method for SLB preparation. We tested the injection of CS, EDC, NHS mixture on the formed SLBs. We used a ratio of 1:2 w/w for CS and both EDC and NH₂, as tested with QCM-D. During the experiment we tested different CS concentrations: 5 mg/ml (as used for the QCM-D experiment) and 25 mg/ml. In both cases we monitored the samples for at least 8h during incubation and collected data before and after rinsing with buffer at pH=5.

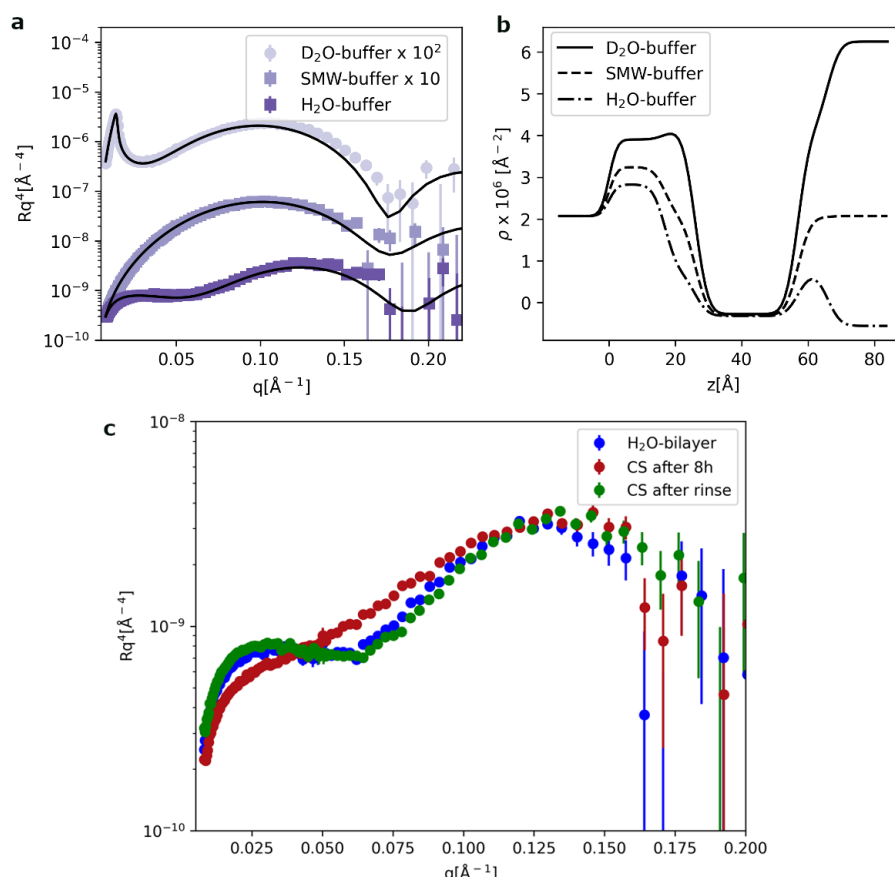


Figure 1: NR data with the corresponding fitting curve (a) and scattering length density profile (b) for the POPC/DOPE-NH₂ (50:50) SLB. Comparison in H₂O-buffer for the SLB before CS injection, 8h after incubation with CS, after rinsing with buffer.

Figure 1c shows the comparison in H₂O-buffer for the POPC/DOPE-NH₂ (50:50) SLB 8h after injection of the solution with CS (25mg/ml), and after rinsing with buffer solution. The injection of CS produced an evident change in the data trend, which is compatible with the formation of a CS layer of approximately 30% surface coverage on top of the SLB. However, upon rinsing with buffer all the initially absorbed CS

molecules are removed. This result was quite surprising given the preliminary QCM-D data that showed no change in the absorbed mass upon rinsing with buffer. We are now working on a more careful interpretation of the QCM-D data. We suspect that also the previously published study on this functionalisation method, which used QCM-D to detect the CS anchoring to the bilayer, might have been affected by a misinterpretation of the QCM-D data. At the same time, we believe a modification of the sample protocol is needed to improve the CS attachment on the bilayer surface. We are currently testing the reaction between CS and DOPE-NH₂ in solution and planning to use IR spectroscopy to detect the amide bond formation. Once we produced the DOPE-NH₂ functionalised with CS, we will use the Langmuir Blodgett- Langmuir Schaeffer deposition to form SLBs with CS exposed on the outer leaflet.

Conclusions. This experiment was the first NR experiment for a newly started project that provided us with unique and crucial information for the design of CS functionalized SLBs. We initially relied on a protocol for sample preparation previously reported in the literature. However, the collected data clearly indicates that modifications of this protocol are required for an effective functionalisation of the SLBs with CS molecules. We are currently establishing this new strategy that we will propose to investigate in a follow-up experiment.

References: [1]Harayama, T. et al., Nat Rev Mol Cell Biol, 19, 281-296, 2018. [2]Oo, Htoo Zarni et al., Cancers, 13, 2021, 13 (4489), 2021. [3]Altgärde, N. et al., JCIS, 390 (1), 258-266, 2013. [4]Ceridório, L.F. et al., Colloids and Surfaces B, 141, 595-601, 2016.[5] Shin, H. et al., Critical Rev in Biochem and Mol Biology, 55(2), 166-178, 2020. [6]Luchini, A. et al., JCIS, 585, 376-385, 2021. [7] Micciulla, S. et al. Biophysical Journal 116, 1259–1269, 2019