Experimental report

30/08/2024

Proposal: 9-11-2128 **Council:** 4/2023

Title: Structure of the ultra-soft interface in aqueous two-phase systems

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: H20
D2O
PEG
Dextran
PEG-deuterated PEG lipids
DSPC phospholipids

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	3	2	18/09/2023	20/09/2023

Abstract:

Aqueous two-phase systems (ATPS) consisting of water and the two incompatible polymers PEG and dextran have numerous applications in the separation and purification of (bio)molecules. The interfaces between the PEG and dextran phases have very low tension and little is known about their structure. Here, we propose the use of neutron reflectometry (NR) for the structural characterization of these ultra-soft interfaces. Because NR from free liquid interfaces of this type is hardly feasible due to high capillary-wave roughness and moderate SLD contrast, we propose an alternative approach based on deuterated PEG polymers attached to the interface between air and a dextran solution. This architecture is a good mimic of ATP interfaces and exhibits low rougness in combination with sufficient SLD contrast.

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In recent years, the study of Aqueous Two-Phase Systems (ATPS) composed of two incompatible polymer solutions has gained significant attention due to their numerous technological applications [1, 3]. Despite its widespread use, little is known about the structure of the polymer-polymer interface on the nanometer scale. Experiment 9-11-2128 was aimed to investigate this interface by neutron reflectometry (NR) at the instrument FIGARO. Of particular interest was the determination of the distribution of the two polymers and water across the interface.
Therefore, we introduced a novel model of the ultra-soft interface between polyethylene glycol (PEG) and dextran by anchoring a PEG polymer brush onto a lipid (1,2-distearoyl-sn-glycero-3-phosphocholine, short: DSPC) Langmuir monolayer at the air/water interface on an aqueous dextran containing subphase. This setup allows the determination of the vertical profile of the system with NR by increasing the accessibility of the PEG/dextran interface while suppressing the interfacial roughness due to the high surface tension of the functionalized air-water interface. The grafting density as well as the length of the anchored PEG polymers (5 kDa) was representative of those in the mimicked PEG bulk phase. Moreover, the extension of the brush (<10 nm) is much smaller than the contour length of the polymers (maximal possible physical extension ≈50 nm) which makes the configurational anisotropy imposed by the end-grafted nature of the brush of minor importance.

Sample preparation
A Langmuir monolayer was prepared by spreading a solution of chloroform containing 10 mol% PEG-lipid and 90 mol% DSPC onto a subphase containing 15 g of dextran per 100 ml of H_2O or D_2O using a Langmuir trough. In order to enhance the neutron scattering length density (SLD) contrast for the PEG chain relative to the subphase and in particular the subphase polymer, we used a custom synthesized PEG-lipid with deuterated PEG chain for all experiments. The system was characterized in two different water contrasts, H_2O and D_2O. The H/D exchange for dextran in D_2O was taken into account. Before the start of an experiment, 15-20 min were allowed for temperature stabilization and solvent evaporation. After solvent evaporation the monolayer was compressed slowly to a surface pressure of 35 mN/m.

Results
To analyze the reflectivity data, a theoretical model system was introduced which allows for the calculation of theoretical reflectivity signals which can be compared with the experimental results. The model describes the volume fractions Φ of all compounds: air, hydrocarbon chain groups (CH), head groups (HG), PEG brush (PEG) and the second polymer (dextran) in subphase (H_2O or D_2O). A number of adjustable parameters is incorporated, which can be fitted simultaneously to the results of the measurement in both water contrasts. Chains and headgroups were modeled by a slab model with constant volume fraction and adjustable thickness, modulated by error functions to account for interfacial roughness, whereas the PEG brush and subphase polymer are modeled by a stretched or compressed exponentional functions. The experimental reflectivity data is shown in Figure 1 (a). The corresponding best fitting volume fraction profile is shown in Figure 1 (b). Figure 1 (c) displays the SLD profiles for both water contrasts.

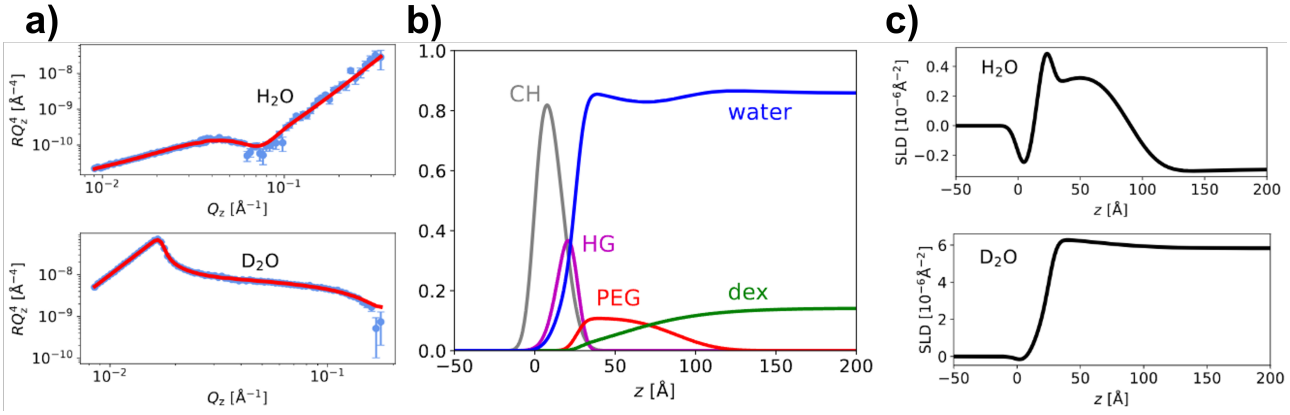

Figure 1: (a) Experimental reflectivity data (symbols) and best matching model fit (lines) for a mixed DSPC/PEG-lipid monolayer at 35 mN/m lateral pressure on aqueous dextran containing subphase in H_2O and D_2O. (b) Corresponding best matching volume fraction profile. (c) Corresponding SLD profiles for the best matching model.

In a second step, the system of a PEG brush was examined on an aqueous PEG containing subphase and on a pure water subphase. In the PEG-PEG case, the SLD contrast was ensured as the PEG brush was deuterated, while the PEG used for the subphase was hydrogenated.
Figure 2 (a) shows the best matching volume fraction profile of a mixed DSPC/PEG-lipid monolayer at 35 mN/m lateral pressure on aqueous (h-)PEG containing subphase as well as the experimental reflectivity data and its fit (solid red line) for both water contrasts. Figure 2 (b) shows the volume fraction profile for the same monolayer on a pure water subphase. In the case of a pure water subphase, just measurements with H_2O were performed.

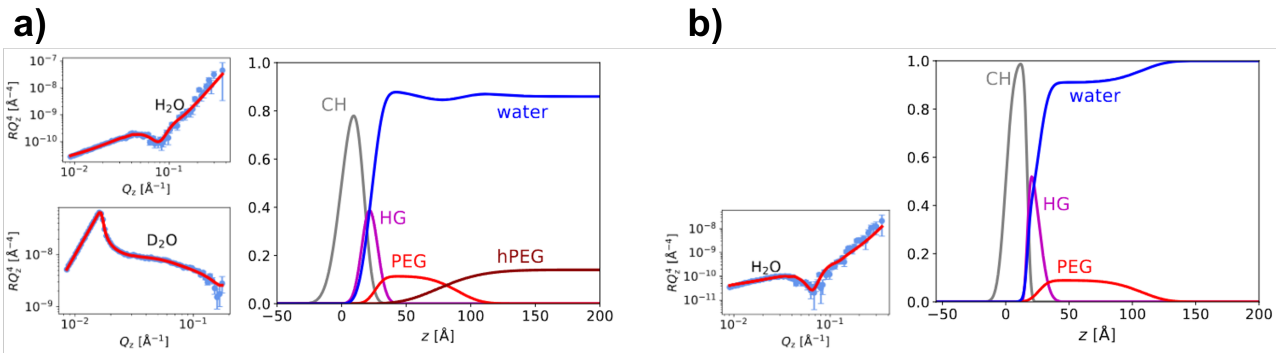

Figure 2: Best matching volume fraction profile of a mixed DSPC/PEG-lipid monolayer at 35 mN/m lateral pressure on aqueous (h-)PEG containing subphase (a) and on pure water (b) corresponding to fit (red solid line) describing the experimental reflectometry data. In the case of a pure water subphase, just measurements on H_2O were performed.

Overall, this NR study has demonstrated that:

1. Our introduced model system proved effective in determining structural details of the PEG-dextran interface by NR.
2. The distributions of the PEG brush and dextran show a significant overlap in the interfacial region. This overlap has been characterized to be in the same order of magnitude in the case of dextran compared to the reference system of PEG, indicating that no clear demixing of PEG and dextran takes place on these length scales.
3. The PEG brush extension was significantly higher in a pure water subphase compared to both polymer containing subphases, indicating a conformational change of the PEG brush due to the competition for water between the brush and the subphase polymer.
4. As an extension to our initial questions, we were also able to determine the volume fraction profiles at different surface pressures, corresponding to different grafting densities. Our data suggests an increase of the PEG chain extension with increasing grafting density as predicted by simple scaling laws like [2, 4].

References

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