Experimental report

12/02/2020

Proposal: DIR-180 **Council:** 4/2019

Title: Caffeine permeability across lipidmembranes

Research area: Chemistry

This proposal is a new proposal

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Samples: synthetic lipids
caffeine

Instrument	Requested days	Allocated days	From	To
D17	2	2	22/01/2020	24/01/2020
FIGARO	2	0		

Abstract:

Passive transport of substances across a lipid membrane is a fundamental biological process with strong implications for medical and pharmaceutical applications. One of the most widely used drug active molecule, caffeine, is a perfect model to understand how purine molecules interacts, cross and accumulate with cell membranes. With this proposal we aim to characterize the kinetics of caffeine absorption in a model bilayer containing a variable amount of polar lipids, mimicking typical composition of PC and PS lipids in mammalian cell membranes.
This experiment will also allow us to locate, with a sub-nanometer resolution, caffeine molecules adsorbed in the bilayer and to monitor structural changes caused by this absorption. Overall, the results will contribute to the understanding of passive transportation of drug molecules and they will show how absorption of caffeine can alter membrane properties and enhance the permeation of other small drug molecules.

Report for DIR-180 on D17

From 22/01/2020 to 24/01/2020

During the experiment DIR-180, we investigated the effect of caffeine on the structure of lipid bilayers for two classes of systems: bilayers pre-loaded with caffeine and pure bilayers exposed to a caffeine bulk solution.

Pre-loaded samples

In order to compare our results with those reported by Khondker *et al.* [1], we used POPC and d_{31}POPC as lipid components. Supported bilayers were prepared on silicon by vesicle fusion. Bilayers pre-loaded with caffeine were prepared by fusion of liposomes prepared with a lipid:caffeine molar ratio of 100:3 and 100:10. The 100:3 ratio was used in [1].

After the characterization of the bare surfaces, samples pre-loaded with caffeine were deposited and measured successfully in four different contrasts. For comparison purposes, bilayers prepared with POPC and d_{31}POPC only were measured in two contrasts.

In Figure 1, the reflectivity curves of a POPC (a) and POPC+Caffeine (3mol%) (c) samples are shown together with the best fits and the corresponding SLD profiles (d,b).

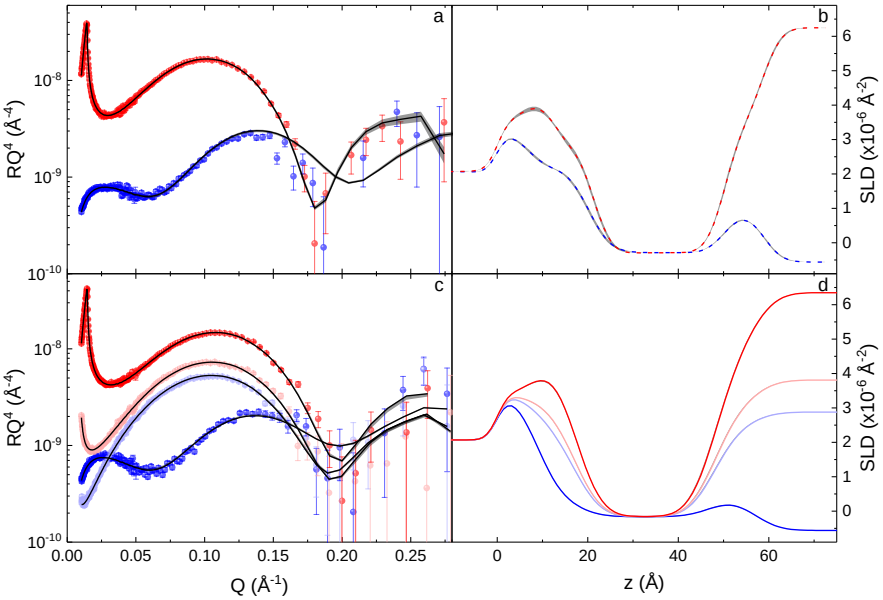

Figure 1. NR curves for a POPC and a POPC+caffeine (3mol%) (a,c) SLB measured in different contrasts. The SLD profiles corresponding to the model curves are shown in b and d with the same colour code used for experimental data. Shaded areas represent the confidence intervals on the models.

Structural differences are present and indicate an increase of hydration in the head-group region and a decrease of hydrophobic thickness upon addition of caffeine. Surprisingly, samples prepared with an higher content of caffeine (10mol%) do not show any difference from the 3mol% samples, indicating a

probable saturation effect. Results were confirmed by additional measurements performed on d_{31}POPC+caffeine bilayers.

Caffeine incubation from solutions

In order to investigate the more relevant interaction of caffeine with model membranes, POPC and POPC:POPS (91:1) samples were deposited on silicon substrates and subsequently exposed to a 0.1 molal solution of caffeine in D_2O. The concentration used was close to the solubility limit of caffeine in water at 20°C (the experiments were performed at 28 °C to avoid problems related to caffeine crystallisation and precipitation). Contrary to previous reports, it was not possible to detect any caffeine molecule within the structure of the bilayers nor structural changes in the bilayers themselves. This might be indicate that, even if caffeine has a large hydrophobic surface, its interaction with phospholipid bilayers (charged or not) is unfavourable in the conditions used during the experiments. Probably other components in the bilayer are needed to promote caffeine adsorption, known to take place in natural conditions. This point will be investigated with complementary techniques before the submission of a continuation proposal.

[1] Khondker, A. *et al.* Phys. Chem. Chem. Phys., 2017, 17, 7101 – 7111, doi : http://dx.doi.org/10.1039/C6CP08104E