ESRF	**Experiment title:** **Hemifluorinated surfactants as a new tool for membrane protein crystallization**	**Experiment number**: MX-1111
Beamline: ID 14-3	**Date of experiment**: from: 24/06/2010 to: 25/06/2010 from 24/02/2011 to 25/02/2011	**Date of report**: 10 10 2011
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Objectives of the study :

The structural resolution of membrane proteins (MPs) at atomic scale encounter a crystallization that is more complicated than that of soluble proteins and still very badly controlled. To rationalize the crystallization process we adopt an original approach with the study of surfactant-surfactant interactions in solution by SAXS, since they seem to control membrane protein interactions during crystallisation (Loll, Pet al. (2002). *Crystal Growth & Design*, Vol. 2 pp. 533-539).

In a previous experiment (Experimental report 20151), dodecylmaltoside (DDM) micelles have been studied because it is the most flequently used detergent for MP manipulation, and it was observed that conditions where interactions in protein free solutions are attractive, are those leading to MP crystallization.

However MPs are often unstable in DDM solution, therefore to overcome this bottleneck, we are developing a new class of surfactants expected to be milder toward MPs, and still able to induce crystallization of membrane proteins. These synthesized surfactants are derived from DDM with the same hydrophilic head and different hydrophobic parts and are described in the proposal report.

Our final objective is to bring more rationality to the crystallization of membrane proteins by studying the implication of news amphiphiles derived from DDM both on structure in solution, assembly, interaction forces and crystallization.

Experimental results :

In this project, SAXS experiments were performed on ID 14-3 at ESRF, to study the behavior of 3 new surfactants in solution, which have been designed by variation of the hydrophobic part in comparison to dodecylmaltoside (DDM) (*see molecules formula in the corresponding application for beam time*). Thus we would like to characterize physical properties differences caused by variations of the hydrophobic moiety. These new surfactants are expected to be interesting tools for membranes proteins crystallization[1]. This is why we focused on the second virial coefficient (A_2) determination to predict their ability to induce MPs crystallization. For each tested conditions, different surfactants amounts (from 2.5 to 50mg/ml) have been used to determined the A_2.

[1] Hovers, J., Potschies, M; Polidori, A; Pucci, B; **Bonneté, F**; Serrano-Vega, M J.; Tate, C G; Picot, D; Pierre, Y; Popot, J-L Nehmé, R; Bidet, M; Mus-Veteau, I; Busskamp, H; Jung, K-H; Marx, A; Timmins, P A; Welte, W
A class of mild surfactants that keep integral membrane proteins water-soluble for functional studies and crystallization
Molecular Membrane Biology 2011 Apr;28(3):171-81

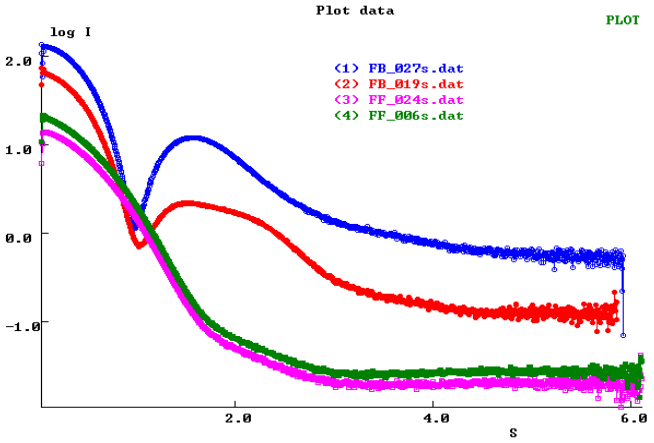

***Figure 1*: Variation of form factor depending on the surfactant**

Surfactant at 10 mg/ml in TRIS buffer, 20mM pH8:

- DDM
- PCC
- F_2H_8
- F_4H_5

Figure 1: First observation deals with the change in the form factor observed for fluorinated surfactants compared to hydrogenated ones

Figure 2 : In a TRIS buffer in absence of precipitant agent, according to determined A_2, F_2H_8 and F_4H_5 are attractive (negative A_2) whereas PCC and DDM are repulsive (positive A_2). However repulsive interactions are stronger for DDM than for PCC (higher A_2). Interestingly this order follows the variation of density of our compounds. Therefore if these results are confirmed, it might point out a very interesting relationship between A_2 and surfactant structure, where the denser the surfactant micelle, the more attractive. Besides, for all surfactants, addition of PEG leads to more attractive interactions, that's why in presence of PEG it is possible to obtain attractive regime with DDM and PCC. However it seems that the strength of the PEG effect depends on the surfactant.

Figure 3: Contrary to PEG, addition of NaCl which is also used as a crystallization agent for MPs doesn't show any effect on A_2 values for PCC and DDM solubilized in TRIS buffer.

Figure 4: Effect of temperature was tested on PCC and DDM in TRIS buffer with 6% PEG, in both case we obtained a smaller A_2 at 10°C than at 20°C, confirming that media are more attractive at low temperatures.

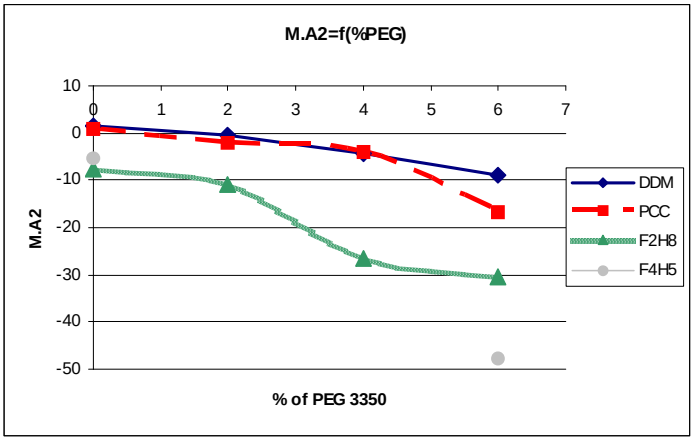

***Figure 2 :* PEG effect on M.A_2 values**

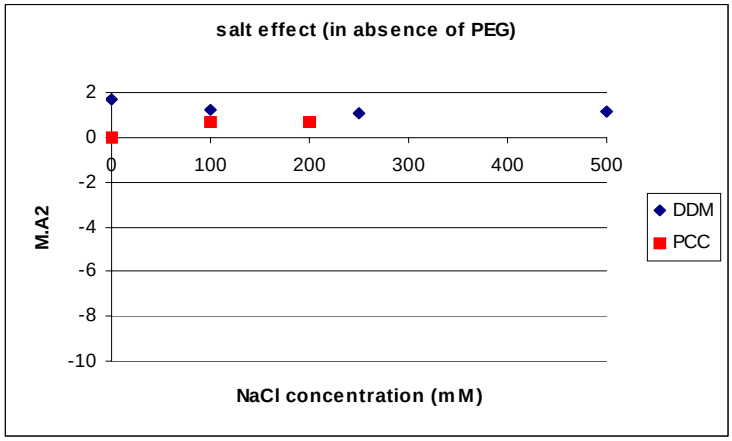

***Figure 3* : Salt effect on M.A2 values**

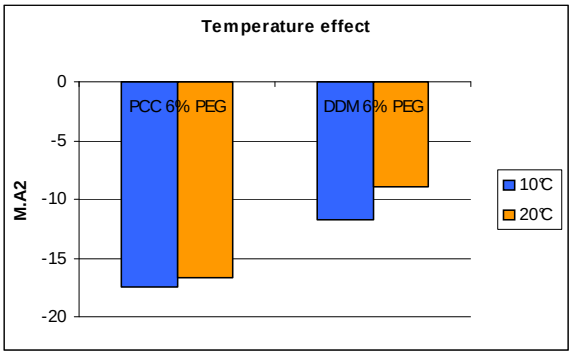

***Figure 4 :* Temperature effect on M.A2 values**