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| ESRF | **Experiment title:**<br>Hemifluorinated surfactants as a new tool for membrane protein crystallization | **Experiment number**:<br>MX1389 |
| **Beamlines**:<br>ID14eh3<br>BM29 | **Date of experiment**:<br>from: 21 /11/2011 to: 22/11/2011<br>from: 15 /06/2012 to: 16/06/2012 | **Date of report**:<br>15/11/2012 |
| **Shifts:**<br>6/6 | **Local contact(s)**:<br>Petra Pernot and Adam Round | *Received at ESRF:* |

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## Report:

In our SAXS experiments performed (since 2010 beginnng of the thesis of L A Barret) at ESRF on beamlines ID14-eh3 then BM29 in 2012, we have studied the behaviour (form factors and structure factors) of new surfactants for membrane protein cristallization (figure 1), designed by variation of the hydrophobic part in comparison to the commonly used dodecylmaltoside (DDM).

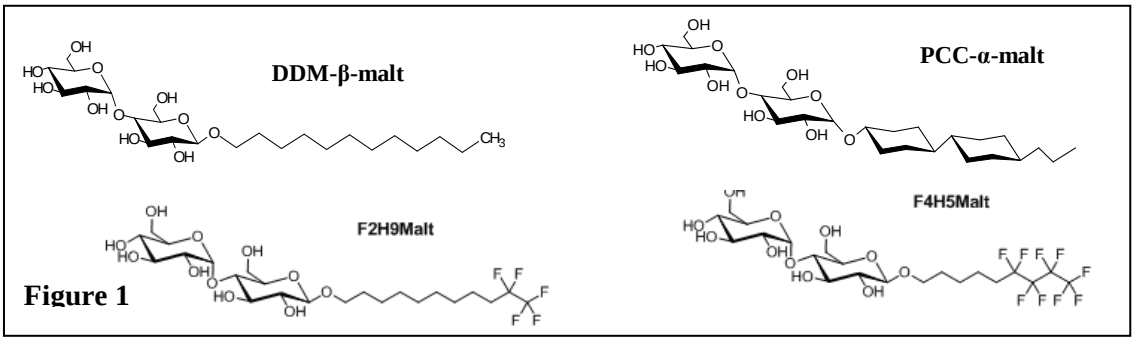


**Figure 1**

DDM and PCC (Hovers *et al, Mol Mem Biol*. 2011, **28:**171) maltosides have been well characterized in term of micelle form factors and their second virial coefficients (characteristic of attractive interactions for successful crystallization) have been evaluated. A paper is currently in preparation for submission in a high impact factor journal (Barret *et al*, *J Phys Chem* B, 2012 to be submitted).

In summary, micelles of DDM and PCC maltoside are quite similar in shape (oblate ellipsoids) but PCC maltoside has a higher aggregation number than DDM ($N_{agg}$ = 160 for PCC vs 125 for DDM) (Figures 2).

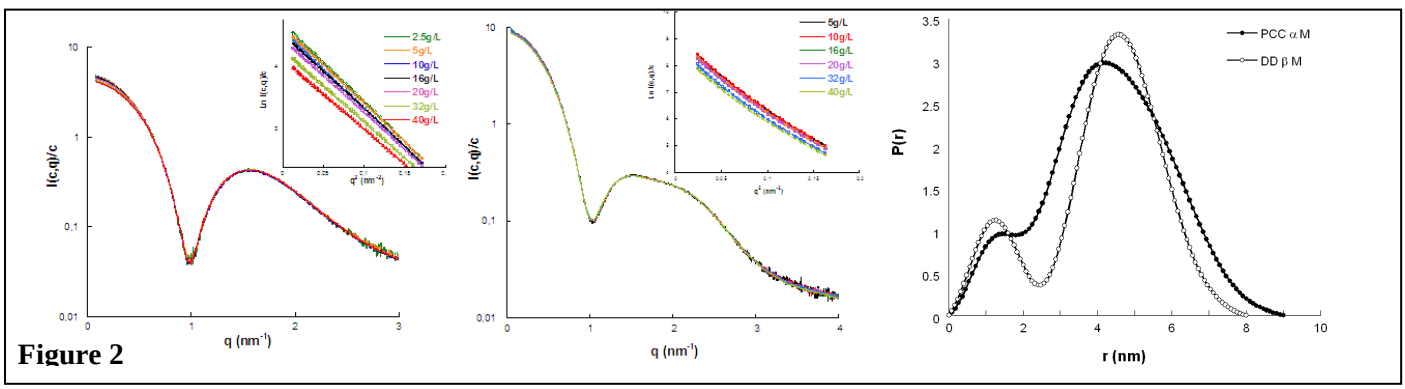


**Figure 2**

The PCC maltoside micelle appears thus denser than DDM micelle, which increases the hydrophobicity and also the van der Waals contribution in the overal interactions between micelles. This increasing

attraction between micelles contributes to the decrease in the consolute boundary of PCC and in RC-LH1-pufX solubility, more favorable to crystallization of the complexe (Figure 3) at lower precipitant agent.

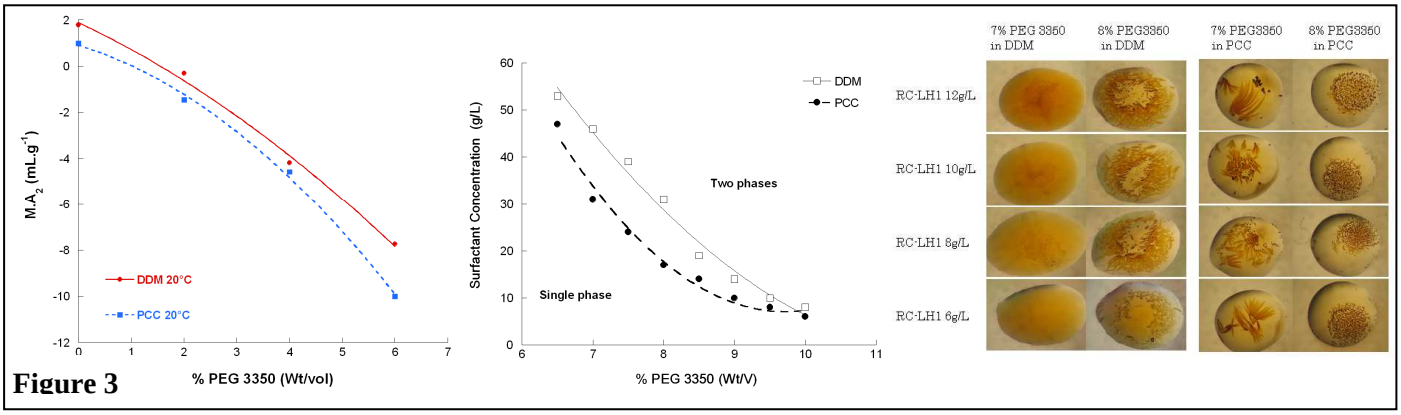


**Figure 3**

In our last experiments at ESRF, we have compared DDM and PCC maltoside with fluorinated parents, $F_2H_9$ and $F_4H_5$ maltoside, in order to compare the steric hindrance brought by a fluorinated chain rather than a cyclic chain (PCC maltoside). The contribution of fluors at the end of the hydrophobic chain modifies FH maltoside form factors as a function of surfactant concentration (figure 4).

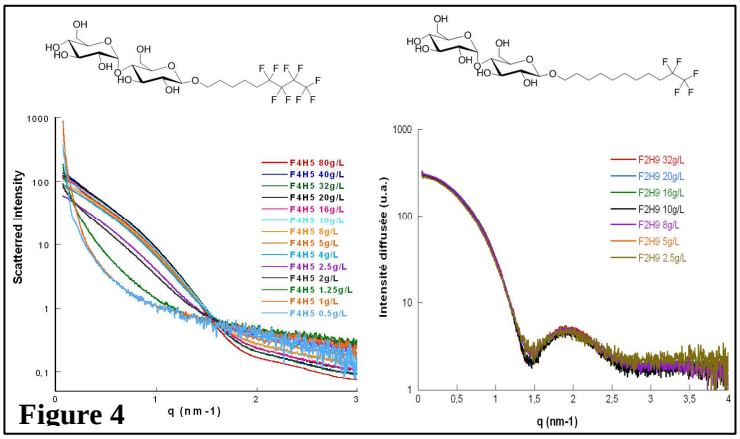


**Figure 4**

It is known that the packing parameter P, which compare polar head area and apolar chain length and volume, permits the control of micelle forms (Israelachvili *et al. J. Chem. Soc. Far. Trans.* 1976, **72**: 1525) (Figure 5).

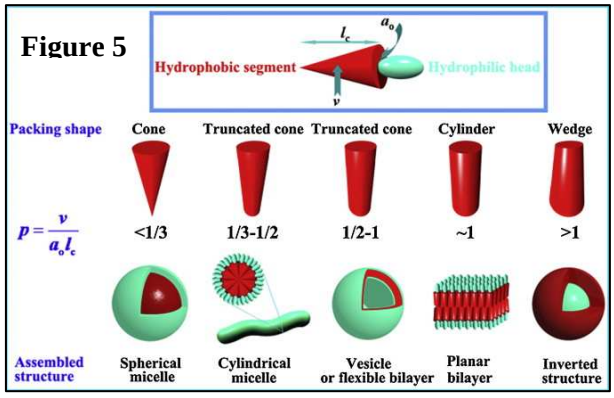


**Figure 5**

It seems that with a maltoside head, micelle lengthens with increasing fluors (F>9), as it was already shown with $HF_6$malt (Polidori *et al*, *Bioorg. & Med. Chem. Lett.*, 2006, 16:5827) . This behaviour has to be compared with another series of fluorinated surfactant, the $F_6$SnGlu series (F=13; n=1,2,3) (Breyton *et al*, *Biophysical J*, 2009, **97**:1077), which shows that a sufficiently large polar head (n=2) is necessary to form spherical micelles with a F6 fluorinated chain. $F_2H_9$ maltoside and $F_6$SDiGlucoside both form small spherical micelles. The stability in the form factor for $F_2H_9$-malt makes us think that second virial coefficient could be measured. Due to some problems with the capillary and troubles during data acquisition in our last allocated beamtime in june 2012 just after the re-opening of BM29, it was not possible to collect satisfactory data for $F_2H_9$malt with addition of crystallizing agent. This experiment has to be performed again in 2013 to finish the works of Laurie Anne barret thesis.

Others questions remain. How many surfactant molecules are bound to a membrane protein in the case of PCC maltoside, $F_2H_9$maltoside and also with $F_6$DiGlucoside? Some membrane proteins have been crystallized with PPC maltoside (Cytochrome $b_6f$ in Hovers *et al* 2011; RC-LH1-pufX our project). Do membrane proteins crystallize with fluorinated surfactants?