ESRF	**Experiment title:** SAXS study of calixarene-detergent/surfactant mixtures for membrane protein solubilization, stabilization and crystallization	**Experiment number:** MX1859
Beamlines: BM29	**Date of experiment:** from: 15/06/2016 to: 16/06/2016	**Date of report:** 29/05/2017
Shifts: 3/3	**Local contact(s):** Petra Pernot	*Received at ESRF:*

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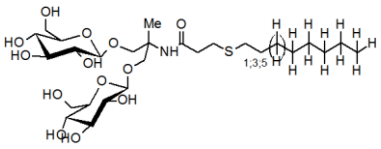
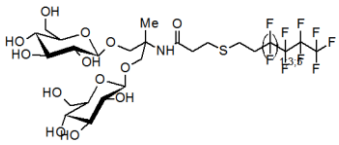
Report:

In this experiment, we have studied two new series of surfactants synthesized in the framework of our LabCom (Chem2staB-Avignon or C2S) for their use in membrane protein (MP) stabilization and crystallization: a calixarene series synthesized and used by Calixar and two diglucoside families, bearing either a hydrocarbonated chain (or a fluorinated chain synthesized by C2S.

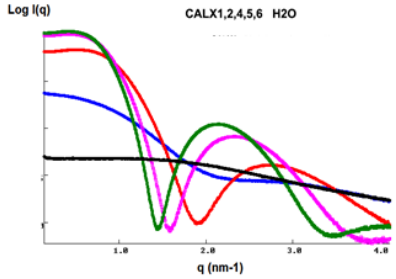




In order to explain extracting/solubilizing and stabilizing/crystallizing properties of these surfactants with membrane proteins, we have characterized the physico-chemical and structural properties of their self-assimblies in solution.

<u>Calixarenes:</u>

CMC of calixarenes C4Cn (n = 3 to 12) have previously been measured by different techniques (fluorimetry, NMR, tensiometry) and have not been checked by SAXS because of the small amounts avaliable. Their capacity either to extract or to solubilize membrane proteins have been shown for calix with n<7 and n> 7 respectively. [1]
Their structures in solution have been studied in order to explain their different behavior with respect to MPs.

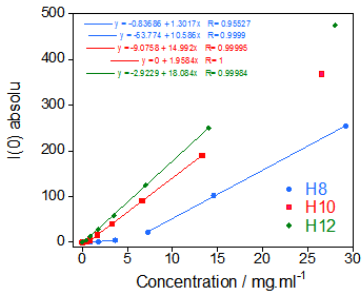


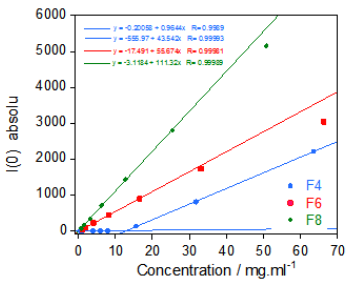
Surfactants	I(0)	Rg (nm) SAXS	Dmax SAXS	Nag SLS/SAXS
Calix 6	9.92	1.6	4.4	~20
Calix 5	20.4	2.02	5.3	~50
Calix 4	23.8	2.21	5.9	~65
Calix 2	2.37	1.2	3.44	1.2
Calix 1	0.58	0.98		1

From SAXS experiments, we observe that Calix C4Cn do not form micelles for n < 7, which can explain their capacity to intrude in membrane and extract MPs but not to solubilize and stabilize them, in contrast with Calix C4Cn (n>7).

<u>Diglu families:</u>

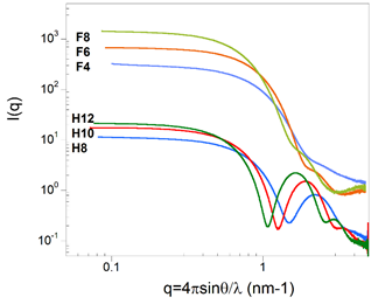
CMC (critical micelle concentration) obtained from SAXS experiments (forward intensity as a function of surfactant concentration) are in good agreement with tension surface measurements (table below)

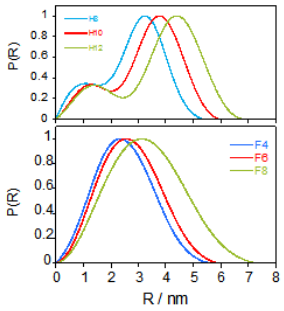




DiGluM	M (g/mol)	SFT (mM)	SAXS (mM)
H8-DigluM	629.76	9.54	9.06
H10-DigluM	657.81	0.55	0.62
H12-DigluM	685.86	0.06	0.14
F4H2-DigluM	763.62	14.4	17.1
F6H2-DigluM	863.63	0.51	0.36
F8H2-DigluM	963.65	0.013	0.029

Self-assemblies formed above CMCs show small globular well defined micelles whose size and aggregation number increase as hydrophobic chain lengthens.





Surfactants	Rh (nm) DLS	Rg (nm) SAXS	Dmax SAXS	Nag
H8-DigluM	2.75	2.22	5.5	26
H10-DigluM	3.28	2.48	6.1	48
H12-DigluM	3.43	2.81	6.9	65
F4H2-DigluM	2.84	2.14	5.7	22
F6H2-DigluM	3.27	2.06	6.0	42
F8H2-DigluM	3.96	2.49	7.4	70

These surfactants therefore present suitable characteristics to be tested in crystallization with membrane proteins. These results have been presented recently [2] and are in preparation for publications.

Références

1. Matar-Merheb, R., et al., *Structuring Detergents for Extracting and Stabilizing Functional Membrane Proteins.* PLOS ONE, 2011. **6**(3): p. e18036.

2. F. Bonneté, G. Nyame Mendendy Boussambe, P. Guillet, A. Marconnet, S. Cassegrain, F. Mahler, C. Vargas, S. Keller, A. Jawhari, G. Durand « *Chem2staB : un laboratoire commun de valorisation de molécules amphiphiles pour la solubilisation, la stabilisation des protéines membranaires* » GdR 3696, Porquerolles 15-18 may 2017.