ESRF	**Experiment title:** SAXS studies of the photosynthetic Light Harvesting complex: implication of protein:lipid:detergent interactions in membrane protein crystallization **Proposal N° 20151**	**Experiment number**:
Beamline: ID14-3	**Date of experiment**: from: 14 05 2009 to 15 05 2009 and from 17 06 2009 to 18 06 2009	**Date of report**:
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Report:

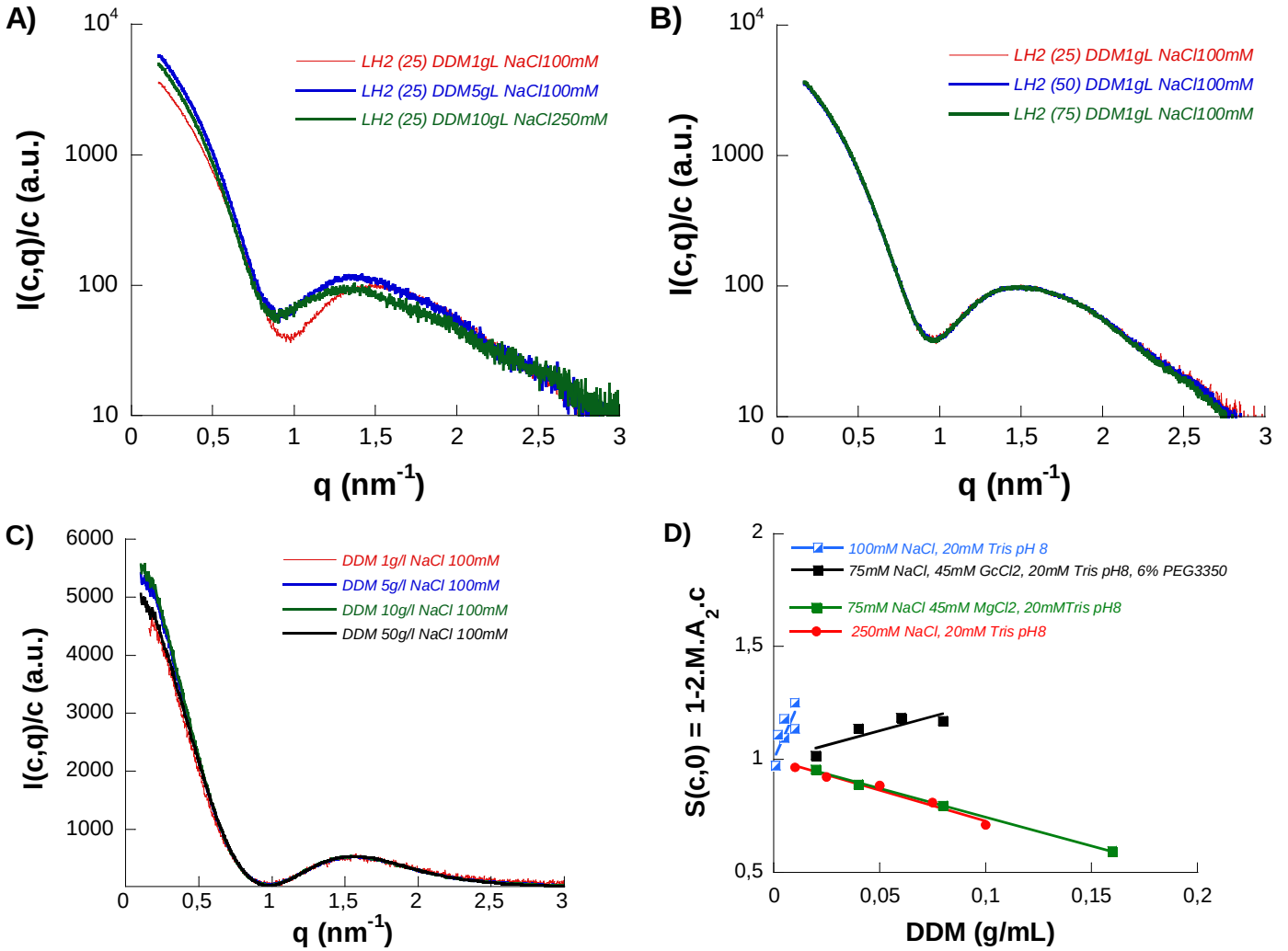
Objectives of the study: Membrane protein (MP) crystallography is challenging because their crystallization is still an empirical process. Difficulties of MP crystallization are mainly due to the specific environment of lipids and detergents surrounding membrane proteins. Rationalization of crystallization is attempted here by an original approach: the study of detergent-detergent interactions which may also control membrane protein interactions for crystallization. Some successful studies have already been done by static light scattering (Loll, 2000; Berger 2006) which show that attractive interactions between detergent micelles can be a predictive tool to predict MP crystallization conditions. However, by using SAXS which give more information than Light scattering, i.e. not only shape information but also structure factor information, we want to go further in the understanding of interaction potentials involved in crystallization mechanism of membrane proteins. The influence of lipid content, the detergent amount bound to proteins, the micelle concentration in the buffer, the nature of detergent, the size of protein in the detergent belt are parameters, which can take part in the different interaction potentials (micelle-micelle, membrane protein complexe-micelle, membrane protein complexe- membrane protein complexe interactions).

Experimental results: In this project, SAXS experiments have been performed on ID14-3 on light harvesting antenna complexes purified in dodecyl maltoside (DDM). Two aspects have been addressed: the amount of detergent used in solubilization of the photosynthetic protein and weak interactions between detergent micelles as a function of precipitant addition.

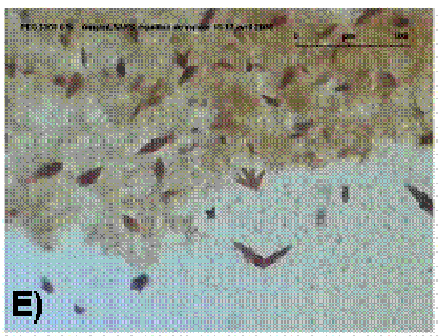
Different amounts of detergent has been used to study the form factor of the membrane protein complex with bound detergent for their influence on MP complexe interaction potentials. A change in the form factors of the photosynthetic complex with increase of

detergent and salt is observed (fig A). Increase in I(0) and Rg as DDM increases are in agrrement with more bound detergent to the protein. However, the concentration of detergent micelles in the substraction buffer has to be precisely determined to correctly define the complex. The use of complementary techniques such as SEC-MALLS apparatus (available in the lab from end 2009) will allow us to characterize the membrane protein complexe in different detergent solutions.

In contrast, no change in intensity is observed as a fct of concentration of membrane protein complexe (Fig B) in each detergent conditions and that, in contrast to detergent micelle behaviour (Fig C). We have characterized attractive ($A_2 < 0$) and repulsive ($A_2 > 0$) interactions between detergent micelles respectively with 100 mM NaCl and 250 mM NaCl (Fig D).



These preliminary results are encouraging since in attractive conditions (6% PEG 3350) between detergent micelles, we were able to obtain crystals of RC-LH1 a photosynthetic complex also purified in DDM (Fig E).



This demonstrates that there is a correlation between crystallization conditions of MP complexe and attractive interactions of the detergent, although there is no significative interactions between MP complexes.

A thorough study of detergent micelle interaction potentiel and MP complexes is therefore necessary to understand MP crystallization mechanisms and the influence of detergent.