ESRF	**Experiment title:** Fusion Stalks in Solution	**Experiment number**: SC4030
Beamline: ID01	**Date of experiment**: from: 01.07.2015 to: 07.07.2015	**Date of report**:
Shifts: 21	**Local contact(s)**: Jan Hilhorst	*Received at ESRF:*

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Report: The GISAXS experiment had two major goals: (1) To extend our previous structural analysis of membrane fusion intermediate structures from protein-free lipid model systems as presented in [1] to a multilamellar system with reconstituted SNARE proteins, following the protein purification and reconstitution protocols presented in [2]. (2) To extend the previous study to highly fusiogenic ("magic") lipid mixtures, which form stalks even in solution (as opposed to the stronger osmotic pressures imposed in humidity cells), based on the phase diagram studies reported in [3]. The investigated sample systems are tabulated in Tab.1.

Tab.1. Sample Systems studied (for lipid acronyms, see also [1,3])

Sample system/ test series	**Lipid Composition / SNARE**	**Preparation**	**Sample Chamber**
PIP2-Series	DOPC/DOPE/PIP2/Chol	Solv.spreading	R.H.
"nature's own fusigenic mixture"	DOPC/DOPE/Chol/Sp.myelin	Solv.spreading	R.H.
Series in PEG solution	DOPC/DOPE/Chol/PIPI2	Solv.spreading	PEG solution
SNARE-mixtures: pure lipid control	DOPC/DOPE/POPS/Chol no proteins	Micelle reconstitution	R.H.
SNARE pre-mixed	DOPC/DOPE/POPS/Chol ΔN+syb	Micelle reconstitution	R.H.
SNARE single component	DOPC/DOPE/POPS/Chol ΔN or syb	Micelle reconstitution	R.H.
SNARE post-mixed	DOPC/DOPE/POPS/Chol ΔN+syb	Micelle reconstitution	R.H.

The setting for the GISAXS experiment at ID01 (see Fig.1) was as follows: 17.81 keV photon energy, 160µm x20µm beam size at the sample, primary beam intensity I=8 10^{10}cps, 15x10 mm^2 silicon wafers carrying a few thousands of oriented lipid membranes in humidity controlled sample chamber, oriented horizontally in the beam by an hexapod positioning system, 4-module Maxipix detector mounted on the detector arm, 182mm behind the sample. For incorporation of the SNARE protein complex, the sample preparation had to be implemented without the use of organic solvents, to avoid protein denaturation. Instead, the SNAREs were reconstituted by surfactant micelles (n-octyl-β-D-glucoside , n-OG), followed by extraction of n-OG in desalting columns, and finally deposition of multilamellar membranes on wafers by vesicle fusion. Surprisingly, this preparation yielded samples which are perfectly suitable for GISAXS, owing to their high orientational alignment.

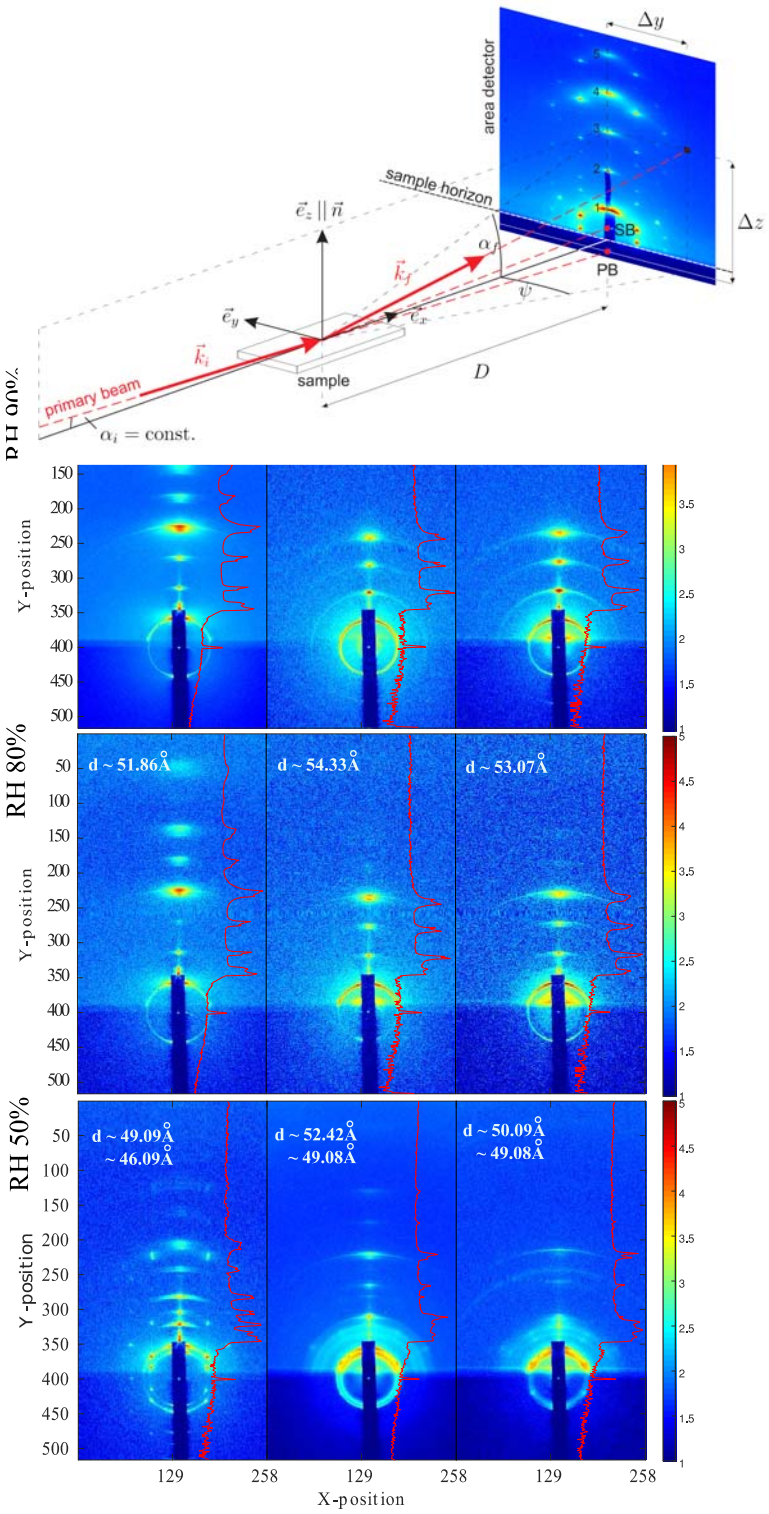

Fig.1: Sketch of the GISAXS geometry of the experiment carried out at the upgrade ID01 beamline, taking advantage of high brilliance, high beam stability and low background to investigate stalk formation with and without membrane fusion proteins.
A total of 37 sample were measured, most of which at many different relative humidity (RH) values, and several different angles of incidence. (Analysis ongoing)

Fig.2 Example of GISAXS diffraction pattern recorded for three different PC/PE/PS/CH samples, with columns representing (left) pure lipid mixture prepared by organic solvent spreading, (center) pure lipid mixture prepared by micellization method and (right) lipid mixture with reconstituted SNAREs prepared by micellization method, respectively. The three rows show data recorded for relative humidities RH 90%, RH 80% and RH 50%, respectively. Corresponding membrane periodicities of the multi-lamellar stacks are marked in the upper left corner of each image.
In general, samples from the solvent method exhibit more Bragg peaks indicative of higher long range order / less lamellar defects. Diffraction patterns of micelle-prepared samples also exhibit high orientation, but more lamellar disorder. The lamellar periodicity also changes, as well as the phase diagram. At RH 50% all samples are in a non-lamellar phase. Only for the pure lipids prepared by the conventional spreading from solvent, the expected rhombohedral (R) phase is observed. The structure changes upon incorporation of the SNARE proteins, but changes in structure are larger between the two different preparation methods.

As the data in Fig.2 shows, the lamellar ordering and phase state differed from samples prepared by the conventional deposition procedure from organic solvent following standard methods for protein-free membranes as used in [1], probably due to the fact that not all of the surfactants can be washed out of the bilayer. While this clearly complicates the ongoing analysis, the high number of control samples will hopefully lead to conclusive results regarding the effect of the SNARE proteins on the phase diagram and stalk structure in the lipid matrix. A second part of the beamtime was dedicated to highly fusiogenic mixtures, where non-lamellar phases were observed under much milder dehydration condition (which were controlled by osmotic stressor solution) than usual (data not shown, also work in progress).

[1] S. Aeffner, T. Reusch, B. Weinhausen, T. Salditt, Proc.Natl.Ac.Sciences 109, 1609-1618 (2012).;
[2] J.M. Hernandez, …, Jahn R et al., Science, 336, 1581-1584 (2012).
[3] Z. Khattari, S. Köhler, Y.Xu, S. Aeffner, T. Salditt, BBA-Biomembranes 1848, 41-50, (2014).