EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

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The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

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Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

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- 1st March for experiments carried out up until June of the previous year;
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Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structure and correlations of membrane fusion intemediates	**Experiment number**: SC-2623
Beamline: ID01	**Date of experiment**: from: May 06th , 2009 to: May 11th , 2009	**Date of report**: March 1st, 2010
Shifts: 15	**Local contact(s):** Dr. Gerardina Carbone, Dr. Peter Boesecke	*Received at ESRF:*

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Report

Peptide model helices of D,L-alternating configuration representing structural analogs of the natural antibiotic gramicidine A (gA) and providing a $\beta^{5.6}$-double helical structure (Fig. 1) were reconstituted in multilamellar lipid stacks of dilauroyl-*sn*-glycero-3- phosphatidylcholine (DLPC, 12:0) and studied with respect to their incorporation, helix orientation and the induced lateral lipid response upon insertion.[1,2] Insertability and orientation according to the membrane normal were, thereby, probed by anomalous scattering in combination with iodine labeling of the incorporated peptide species; the impact of peptide insertion on the peptide-lipid interactions within the membrane plane was addressed via Gracing Incidence Small Angle X-ray Scattering (GISAXS).[3,4]

Regarding the analysed peptide species, the focus of the anomalous reflectivity experiments laid on validating the ability of recently developed hairpin structures (**1**) to function as membrane spanning anchors in a dynamic, peptide-based model recognition machinery at the membrane/water interface (Fig. 1).[5]

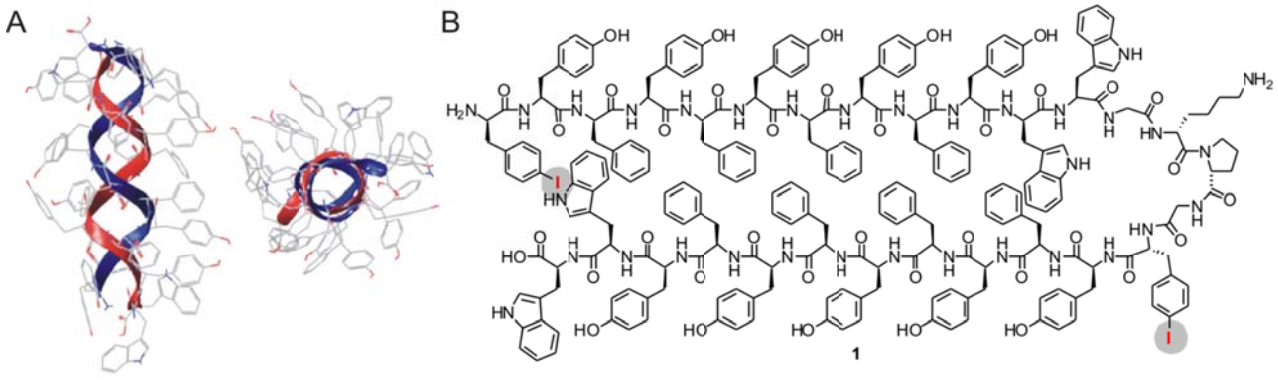

Fig. 1. Molecular structure of the $\beta^{5.6}$-double helical homodimer version (A) and molecular representation of a double iodine labeled hairpin species **1** (B) applied in the anomalous reflectivity experiments.[2]

Therefore, a detection of the iodinated amino acid side chains related to the membrane normal (*z*-axis) was performed applying reflectivity experiments close and afar the iodine L_{III} absorption edge at E = 4.5578 keV and E = 5.8000 keV respectively (Fig. 2). The electron density profiles were derived via *Fourier* synthesis taking into account the integrated intensities of the *Bragg* reflexes. Maxima in the associated difference curves indicate the position and depth of the certain iodine atoms within the lipid bilayer (Fig. 2).[6] The analyses of three differentially labeled peptide species via anomalous scattering and in-house reference experiments provided strong evidence for an deep insertion of the peptide helices into the lipid matrix concordant with adoption of a membrane (hydrophobic core) spanning orientation.

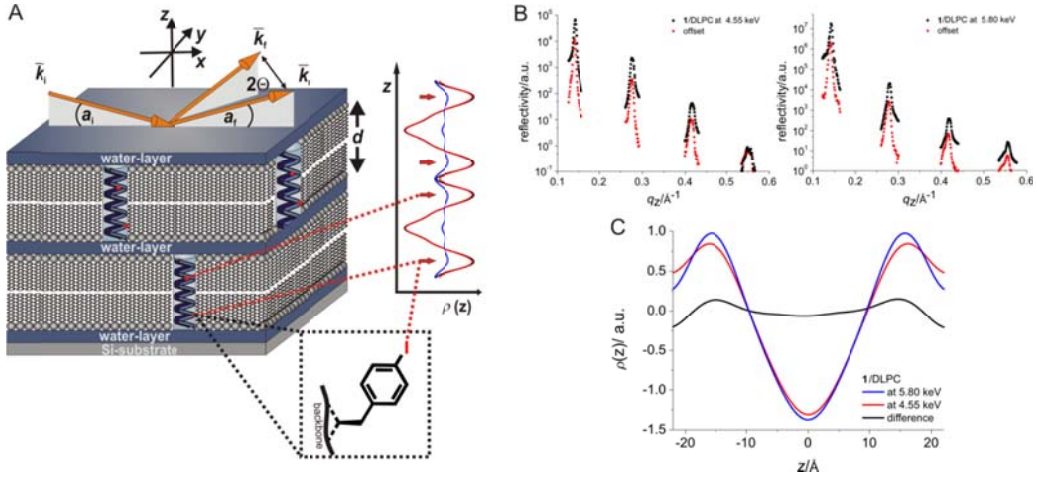

Fig. 2. Scattering geometry of the reflectivity experiments (A) and exemplaric reflectivity curves obtained at the different photon energies (C) leading to the reconstructed electron density profiles and the repective difference curve (C).

Since highly oriented lipid films were analysed in the GISAXS experiments, the electron density is isotropic in the x-y-plane and the reciprocal space appears two-dimensional defined by the q_z and the $q_{||}$ axes (Fig. 2). Diffraction pattern were recorded with a CCD detector (1340 × 1300 pixels) at a sample-to-detector distance of typically 19 cm applying a controlled relative humidity (*RH*). The deduced reciprocal space mappings (RSMs, Fig. 3) provided the lipid chain correlation peak that is stretched along a circle with the radius $q = (q_{||}^2 + q_z^2)^{1/2}$ Å. From *Lorentzian* fitting the chain correlation analysis yielded the lipid acyl chain distance as the repective parameter in real space to estimate the magnitude of peptide lipid interaction. The comparison of D,L-alternating homodimeric (hd) peptide species, peptide hairpins (hp) like compound **1** and pairing peptide-peptide nucleic acid (PNA) conjugates at various *RH* let us propose a qualitative model peptide-lipid interactions that is characterized by an annular lipid shell providing enhanced lipid packing, acyl chain stretching in z-direction and lipid ordering. In some way contradictory with respect to models reported in the literature these observations may be attributable to the extraordinary and solely aromatic side chain composition of the considered peptide sequences.[7,8]

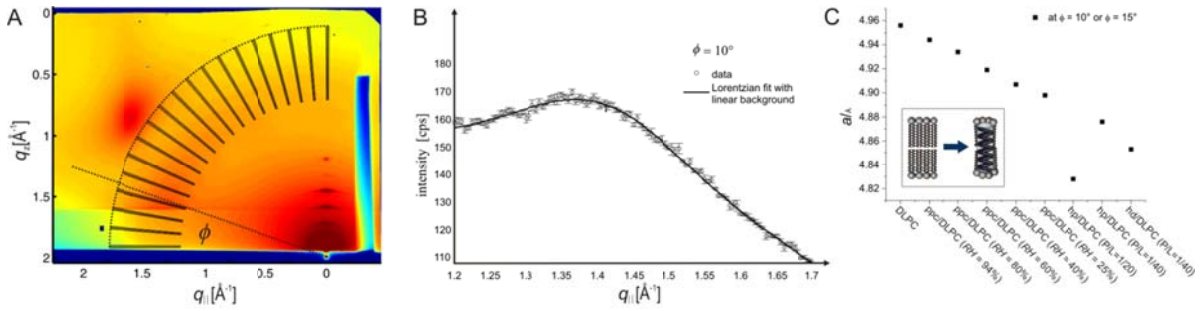

Fig. 2. Exemplaric RSM (A) indicating several sections through the reciprocal space leading to the fit of the chain-chain correlation peak at low ϕ (B) that gives the lipid acyl chain distance (C). The values of the different peptide species suggest an increased packing shell of the lipid molecules in close proximity to the incorporated peptide species (C).

References

[1] E. Alexopoulos, A. Küsel, G. M. Sheldrick, U. Diederichsen, I. Usón, *Acta Chrystallogr.* **2004**, *D60*, 1971-1980. [2] A. Küsel, Z. Khattari, P. E. Schneggenburger, A. Banerjee, T. Salditt, U. Diederichsen, *ChemPhysChem* **2007**, *8*, 2336-2343. [3] E. Arbely, Z. Khattari, G. Brotons, M. Akkawi, T. Salditt, I. Arkin, *J. Mol. Biol.* **2004**, *341*, 769-779. [4] D. Constantin, G. Brotons, A. Jarre, C. Li, T. Salditt, *Biophys. J.* **2007**, *92*, 3978-3987. [5] P. E. Schneggenburger, S. Müllar, B. Worbs, C. Steinem, U. Diederichsen, *J. Am. Chem. Soc.*, submitted. [6] P. E. Schneggenburger, A. Beerlink, B. Worbs, T. Salditt, U. Diederichsen, *ChemPhysChem* **2009**, *10*, 1567-1576. [7] P. Lagüe, M. J. Zuckermann, B. Roux, *Biophys. J.* **2001**, *81*, 276-284. [8] H. Palsdottir, C. Hunte, *Biochim. Biophys. Acta* **2004**, *1666*, 2-18.