ESRF	**Experiment title: Conformation and interaction of ZipA: an essential inner membrane *E. coli* protein**	**Experiment number**: SC-2876
Beamline: ID02	**Date of experiment**: from: 05/03/2010 to: 08/03/2010	**Date of report**: 13/10/2014
Shifts: 9	**Local contact(s)**: Jérémie Gummel	*Received at ESRF:*

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Report:

The purpose of this experiment was to determine the in-plane interaction of the intrinsically disordered protein ZipA (integral to the inner membrane of *E. coli*) inserted in lipid bilayers, under temperature and concentration conditions similar to its physiological environment in the septal ring, the bacterial structure that initiates cell division.

Experimental

The small-angle X-ray scattering measurements were performed at the ID02 beamline, in a SAXS+WAXS configuration, at an X-ray energy of 12.4 keV ($\lambda = 1$ Å). For the SAXS detector we used two sample-to-detector distances: 1 m and 2.5 m, for an accessible q-range of 0.1 to 6 nm^{-1} and 0.035 to 3 nm^{-1}, respectively. The WAXS range was 5 to 53 nm^{-1}. The samples were prepared by mixing appropriate amounts of ZipA protein and lipid (*E. Coli* extract or a similar mixture of synthetic lipids) at the desired molar ratio P/L (peptide/lipid), hydrated in excess solvent and filled into flat glass capillaries (with section 0.1 × 1 mm^2 and a wall thickness of 0.1 mm.)

Unfortunately, we encountered two experimental problems when preparing the protein/lipid samples: first of all, we weren't able to obtain good alignment of the lipid bilayers with respect to the walls of the flat cells. Second, even for the (very few) well-aligned samples, the signal was not reproducible, see the example in Figure 1.

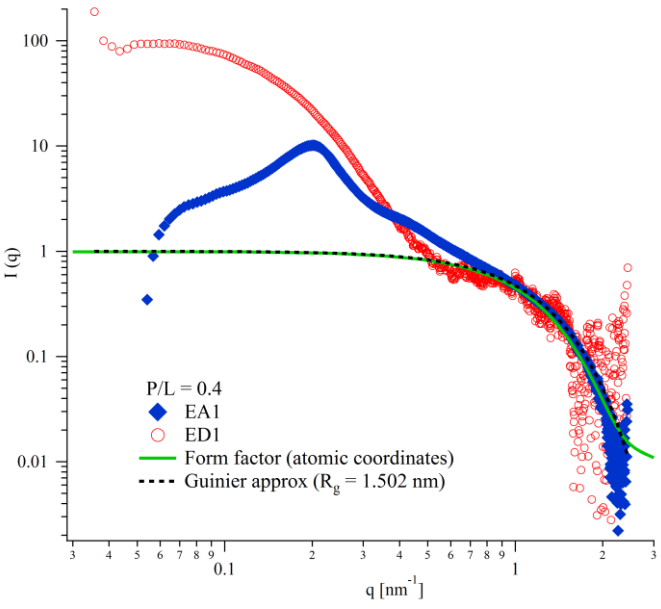

Figure 1: In-plane signal for two samples, with the same nominal concentration P/L (symbols). Calculated form factor of the folded domain at the C-terminus (solid line) and its Guinier approximation.

We can, however, draw two interesting conclusions from the data shown in this Figure:

- The measured signal is likely due to the (hydrophilic) folded domain at the C-terminus, since the high-q part of $I(q)$ agrees very well with the form factor of this region, calculated from the atomic coordinates [1]. This result is expected, since the folded domain is the only compact region of the protein.
- In well-homogenized samples (curve EA1, blue diamonds in Figure 1), an interaction peak is clearly visible around 0.2 nm^{-1}, while the small-angle increase is very moderate, showing that there is little or no aggregation, in contrast with other –presumably heterogeneous– samples (red dots).

Since the problems mentioned above rendered this system very proving difficult to study, at the end of the run we switched to a backup system: lipid bilayers doped with the antimicrobial polypeptide gramicidin. In particular, we measured the shape of the lipid chains, available in the WAXS domain, as a function of the gramicidin content (expressed as the molar ratio P/L) and temperature. The HWHM of the peak is plotted in Figure 2.

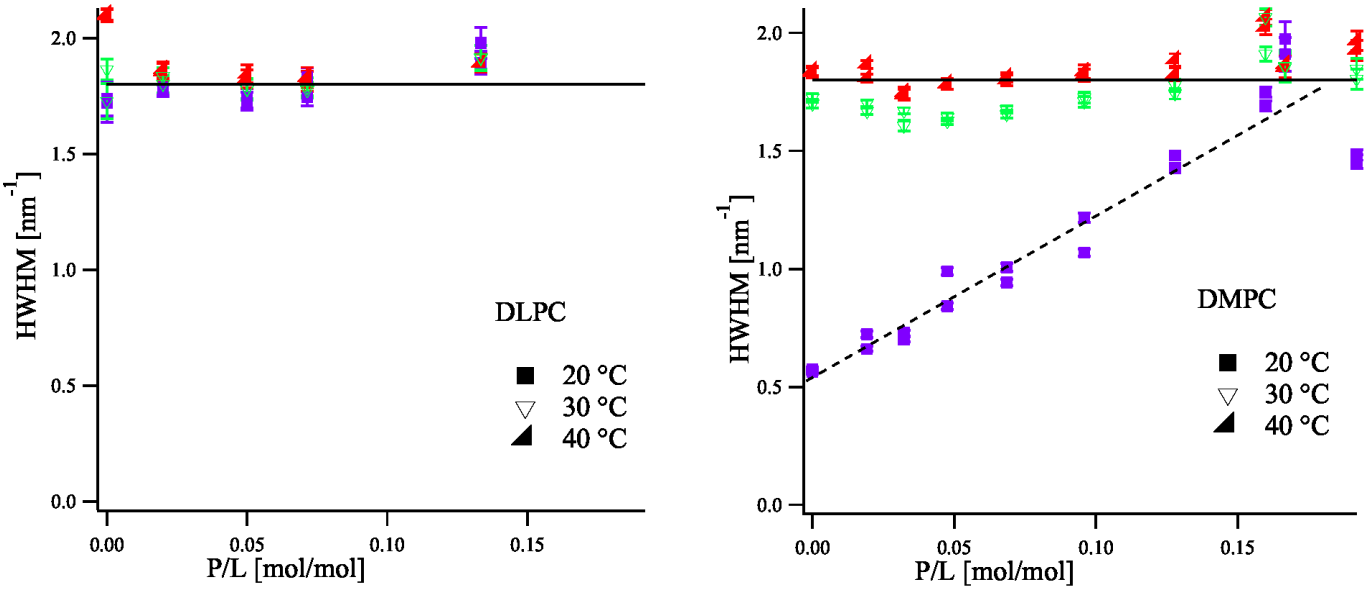

Figure 2: Width of the chain peak for DLPC (left) and DMPC (right) bilayers as a function of the gramicidin doping at three temperatures.

Conclusions

This study provided the proof of concept that the signal of the C-terminus domain of ZipA can be detected when the protein is inserted within lipid bilayers and that it exhibits an interaction peak. We are therefore confident that quantitative measurements of the protein-protein interaction will be possible once we achieve the preparation of homogeneous and well-aligned samples of lipid lamellar phases.

In the gramicidin/lipid system we were able to measure precisely the chain peak. An interesting feature is that in DMPC at 20°C (in the gel phase) the peak is very sharp in the absence of gramicidin (as expected) but widens with increasing doping and reaches the width measured in the high-temperature liquid phase for P/L ~ 0.2. This effect will be compared with NMR results for the order parameter of the chains and should provide insight into the influence of membrane proteins on the state of the neighboring lipid molecules. We will also attempt a full-shape analysis of the chain peak [2].

References

[1] F. J. Moy et al., Solution Structure of ZipA, a Crucial Component of *Escherichia coli* Cell Division, *Biochemistry* **39**, 9146 (2000).

[2] T. T. Mills et al., Order Parameters and Areas in Fluid-Phase Oriented Lipid Membranes Using Wide Angle X-Ray Scattering, *Biophysical Journal* **95**, 669 (2008).