ESRF	**Experiment title:** Characterization of intra- and intermolecular structure of NLPs in the presence of plasma membranes by SAXS	**Experiment number**: SC-3274
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Report:

NLPs are proteins with necrotizing activity on dicot plants, which features them as important virulence factors in necrotrophic pathogens [1-3]. As the attack of these hosts leads to tremendous damage on economic plants, the elucidation of the molecular mode of action of NLPs is of enormous interest. We have demonstrated that the necrotizing activity of NLPs depends on a lysis of the plasma membrane in dicot plants [2]. Our success in resolving the crystal structure of an NLP from *Pythium aphanidermatum* revealed structural similarities to pore forming toxins from marine invertebrates. A well-examinated example of this protein family is Sticholysin, which binds to membranes via a sphingomyelin specific binding site [4]. Upon membrane contact, an N-terminal helix is elongated and inserted into the membrane. The helixes of four toxin molecules form a transmembrane pore under participation of lipids. The structural similarity of NLPs to pore forming toxins together with their cytolytic activity suggests that the NLP activity is based on a similar mechanism. NLPs might bind to a plasma membrane component and impair the integrity of the PMV by forming transmembrane structures. Latest experiments with protease treated plasma membrane, which are still sensitive to NLP, leads to the presumption that the target molecule is probably of lipid-like nature.

The aim of the project is to elucidate how NLP molecules behave upon contact with plasma membranes. In this context, we have performed DLS measurements on the proposed systems, which give a narrow distribution of PMV before and after proteinase treatment with an average hydrodynamic radius of 30 nm. Upon addition of wt-NLP, the overall size of PMV increases gradually to about 50 nm within 4 hours. As the NLP sensitivity is restricted to dicot plants, but the target molecule is unknown, the experiment has to be carried out on purified dicot plasma membranes instead of ordinary lipid vesicles. In the case of promising data, the behavior of inactive mutants of the protein should be tested as well as the effect on plasma membranes from resistant organisms.

In this beamtime we measured the solution scattering of the pure protein wild type wt-NLP and two mutated protein versions NLP H101A (inactive) and NLP D104A (intermediately active) in solution by

SAXS as a function of protein concentration (0.1 to 5.0 mg/mL). The pure plasma membrane vesicle (PMV) solutions with and without protease treatment are also measured by SAXS in order to determine the size, shape and the basic structure of the vesicles as a function of plasma concentration. Small-angle X-ray scattering (SAXS) measurements were carried out at station ID2, ESRF, Grenoble. The energy of incoming beam was 16 keV, two sample-to-detector distances (2 m and 5 m) were used in order to cover a large *q* range from 0.02 to 7.8 nm^{-1}. Protein solutions were filled into 2 mm quartz capillaries. The same buffer solution without protein was measured as the background, in exactly the same way as the protein solutions and was subtracted from the sample scattering. All measurements were carried out at room temperature. The raw data were corrected for transmission, fluctuation of primary beam intensity, exposure time, and the response of the detector [5,6].

Fig.1 (left) shows the SAXS data for wild type NLP and mutant NLP in solution. The intensity values are shifted for clarify. Visible difference can be seen in the intermediate q range, where the scattering intensity decays dramatically with q. For wild type NLP the decay is faster than the mutant type of protein, indicating the partly open structure of protein after mutation. This structure change may be related to their activity to the PMV. Detailed structural analysis using *ab initio* method will be followed. Fig.1 (right) shows the scattering curve of pure PMV. The data are merged from two SD setups. Interestingly, the data are well described using a core-shell oblate model instead of a core-shell sphere model. This result suggests that the vesicles have a rather oblate like shape instead of the spherical shape. Our further study will focus on the structure of the complex of PMV with different types of NLP, which will provide insight of the mechanism of the activity of wild type NLP on PMV.

In summary, we have shown that the wild type and mutant NLP in solutions have different scattering patterns, indicating the possible conformation change of protein in solution. The scattering curve of the plasma membrane vesicle, PMV, can be well described using a core-shell model. These results make it possible to perform a study on the dynamic procedure of NLP attachment on the PMV and the structure change of the complex, which we plan to do in next beamtime.

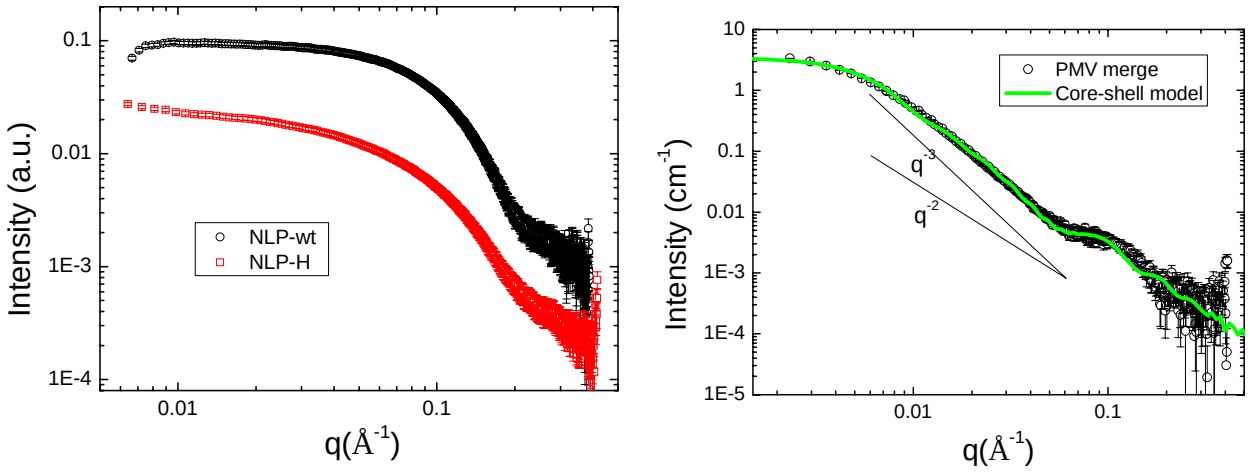

Figure 1. (left) SAXS profiles of wild type (NLP-wt) and mutant NLP (NLP-H101A) and (right) the scattering of PMV with model fitting. Data are collected with two SD configurations (2 and 5 m).

References

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