ESRF	**Experiment Title:** Solution structure of nucleases from three staphylococcal species and the high temperature and salt tolerance by SAXS	**Experiment number**: MX2510
Beamline: BM29	**Date of experiment**: from: 6th April 2023 to: 8th April 2023	**Date of report**: 5th Dec. 2023
Shifts: 6	**Local contact(s):** Dr. Petra Pernot	*Received at ESRF:*

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Report:

As viral therapy technologies have advanced, the need to develop reliable production strategies has grown. For example, many promising gene-therapy strategies use adeno-associated virus (AAV) or other viruses as a vector. However, a challenge in the production of viruses is residual DNA released from the host cell. An effective strategy for reducing residual DNA is to use a nuclease. Since high levels of NaCl are often used to improve recovery during AAV purification it is essential that such biotechnologically used nucleases are salt tolerant (0.5 NaCl) and active at 25 to 40ºC [1]. We have cloned two nucleases from *Staphylococcus chromogenes* which is one of the main coagulase-negative staphylococci isolated from mastitis of dairy cows [2]. This species contains two nucleases Nuc19 (19 kDa) and Nuc13 (13 kDa). While Nuc19 is secreted and has a signal peptide (SP), Nuc13 has no SP and is therefore cytoplasmic localized.

Using NucA, a well-studied nuclease, as a reference, the goal of this proposal is to characterize the three Nucleases systems (NucA, Nuc13 and Nuc19) for their stability under the crucial application conditions including salt tolerance and high temperature using SAXS. Previous work has shown that NucA and Nuc19 are highly stable, whereas Nuc13 form oligomer or clusters overtime.

During this beamtime at BM29, we measured the three nuclease systems for 5 different concentrations ranging from 1 mg/ml to 10 mg/ml, and 5 temperatures up to 50 ºC, and proteins with different salt concentrations (NaCl), buffer and co-factors. Most of the measurements were successful and the data are in high quality. One example shown in Fig.1 is the scattering profiles of Nuc19 in PBS buffer for different protein

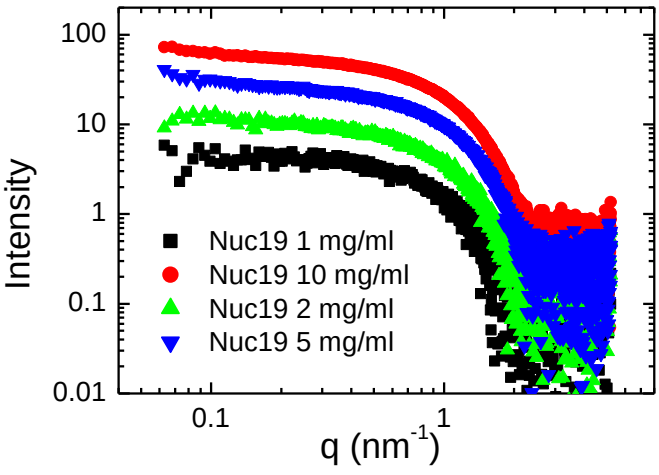

Figure 1: SAXS profiles of Nuc19 in PBS buffer for different protein concentrations.

concentrations. These high-quality data will be further analysed to obtain the information of protein size (radius of gyration, Rg) and molecular weight as a function of protein concentration, temperature, salt concentration, to reveal their stability and tolerance with salt.

The broad biotechnological application of nucleases requires high salt tolerance and high temperature stability. The structural study of this work for three staphylococcal nucleases with different primary sequences together with their activity property determined in our laboratory will provide crucial information about the relation between the primary structure and its properties. We are currently preparing a manuscript based on the SAXS data collected in this beamtime.

Furthermore, we have used this opportunity to measure a series samples of protein-polyelectrolyte coacrevates, i.e. BSA with PDADMAC. The mixtures undergo a liquid-liquid phase separation, and this system can be used as a model system for the understanding of the structure, interaction, and partitioning of both protein and polyelectrolytes within the biological condensates. Further studies and research will be proposed based on these test results for further beamtime applications.

References

[1] Adams, B., H. Bak & A. D. Tustian, (2020) *Biotechnol Bioeng* **117**: 3199-3211.

[2] Dos Santos, D. C., C. C. Lange, P. Avellar-Costa, K. R. Dos Santos, M. A. Brito & M. Giambiagi-deMarval, (2016) *J Clin Microbiol* **54**: 1372-1375.