ESRF	**Experiment title:** **Antimicrobial Peptide-polymer coacarvate micelles: shining light on the kinetic pathway of formation**	**Experiment number**: SC-5313
Beamline: ID02	**Date of experiment**: from: 27.09.22 to: 30.09.22	**Date of report**: 13-02-23
Shifts: 9	**Local contact(s)**: Lauren Matthews	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Reidar Lund, Department of Chemistry, University of Oslo*

Vladimir Koynarev, Department of Chemistry, University of Oslo*

Ola Krogseth, Department of Chemistry, University of Oslo*

Henrik Torjusen, Department of Chemistry, University of Oslo*

Szymon Szostak, Department of Chemistry, University of Oslo*

Thomas Vogelaar, Department of Chemistry, University of Oslo*

Josefine Nielsen, Department of Science and Environment, Roskilde University*

Report:

The beam time at the end of September was based on the proposal that was sent in February 2022. In the proposal, we proposed a series of Time-Resolved X-ray scattering experiments using the Stopped-Flow to reveal the coacervation mechanism of mixing PEO-PMAA polymer with two different antimicrobial peptides with therapeutic potential: LL-37 and indolicidin. During the beam time, our focus was to gather systematic data (different charge ratios to discover the principles of coacervation for this specific system) on a different antimicrobial peptide: colistin, as we found previously that LL-37 and Indolicidin were less interesting to systematically study. Approximately two-thirds of the shifts were spent on this.

We got great insights into how these complexes are formed at different charges, and what factors of the electrochemical screening from the salt are important in their formation. The kinetic rate constants will be determined as data for all charge ratios at three different concentrations was gathered.

After preliminary analysis, we already got a good understanding of coacervation, which will lead to some progress in being able to use antimicrobial peptides for therapeutic use. A paper will be written based on this experiment. In Figure 1, some preliminary 3D plots in which the progress of the coacervation can be followed over time for charge matching conditions. Three features change over time after the components have been mixed (Fig. 1). An increase at low Q can be observed, indicating growth of the micelles, a steeper curve at medium Q, including a shoulder formation, and an increase in the bump at high Q, indicating a preferred spacing pattern for the components in the coacervates. The same observations were found for all charge ratios and did not seem concentration dependent.

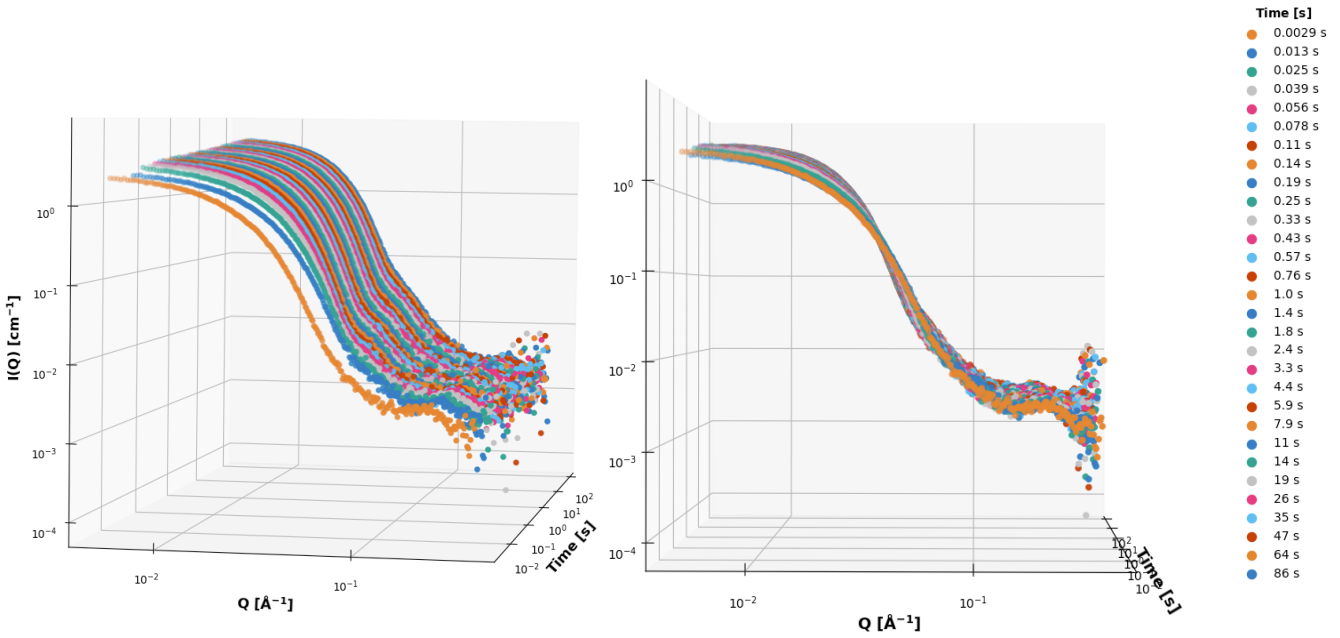

Figure 1: SAXS curves over time from two different angles on the formation of PEO-PMAA:Colistin complex coacervate micelles at charge matching conditions at ID02.

In the time we had left, some other experiments were performed. Peptide-peptide interactions, wide-range temperature scans on self-assembled peptoid structures, and the remaining hours were spent on the disruption of liposomes after the stopped-flow mixing with the antimicrobial peptide LL-37. Especially the disruption of the liposomes seemed promising to systematically study further (Fig. 2).

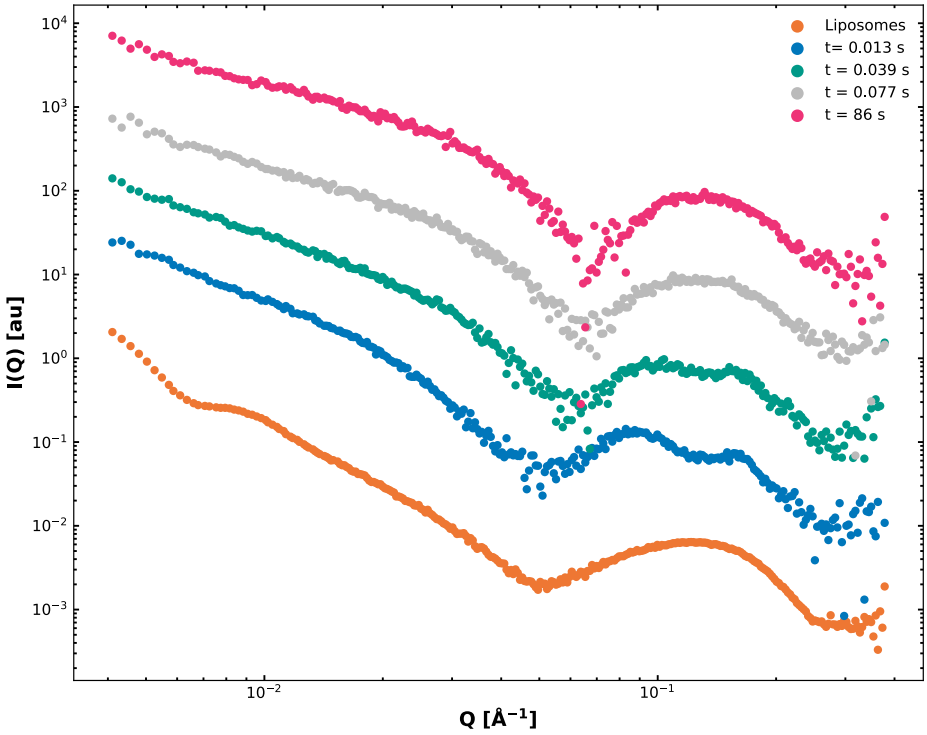

Figure 2: SAXS curves over time from ID02, following the Liposome disruption by LL-37. The data is on absolute scale but with factors of 10 to increase visibility of the changes in structure.

The minima appear to shift to the right, as well as some intermediate bumps created at high Q (Fig. 2). There appear to be big rearrangements from liposomes to unknown structures, which have to be studied further to create a better understanding of the interactions between model membranes and antimicrobial peptides. We will apply in the next cycle for a beam time to get to study these systematically, and potentially also include other antimicrobial peptides.