Experimental report

01/09/2022

Proposal: 8-04-929 **Council:** 4/2021

Title: Towards a microscopic picture of coacervation in elastin-like polypeptides

Research area: Biology

This proposal is a new proposal

Main proposer: Felix ROOSEN RUNGE

Experimental team: Sarah WALDIE
Tatiana MOROZOVA
Zeina WEHBE

Local contacts: Olga MATSARSKAIA
Michael Marek KOZA

Samples: elastin-like peptides in D2O buffer

Instrument	Requested days	Allocated days	From	To
IN5	3	3	20/09/2021	23/09/2021
D11	2	1	23/09/2021	24/09/2021

Abstract:

We aim for a comprehensive understanding of the hydrophobic collapse and the related coacervation of elastin-like polypeptides (ELPs) by combining structural and dynamical signatures using SANS (D11) and QENS (IN5), complemented by laboratory SAXS, light scattering, CD spectroscopy, and computer simulations. Using protonated and perdeuterated versions of the ELP GVG(VPGVG)42, we address the dynamical nature and conformational state of ELPs in coacervates.
The studied system not only provides a model system for the hydrophobic segments of elastin which are relevant for the extraordinary mechanical properties in biology and elastin-based biomaterials. In addition, the results pave the way for future research using cellular peptides with similar phenomenology.
The project is based on an established collaboration between Malmö University and the ILL (LSS group, D-Lab and theory group). The recombinant production of protonated and perdeuterated ELPs has been successfully established in the D-Lab, and sufficient material for this proposal has been expressed. The collaboration has ample expertise regarding the characterization of dynamics and structure in protein solutions and assemblies

Experimental report - 8-04-929 (D11 and IN5, 23-24/09/2021 and 20-23/09/2021)
Structure and dynamics of elastin-like peptides upon hydrophobic collapse

Scientific background. Due to their stimuli-responsive properties, elastin-like peptides (ELPs) have been increasingly used in a broad range of applications including biomaterials, protein purification and drug delivery during the last two decades. ELPs are artificially designed biomolecules mimicking the hydrophobic repeat units in elastin, an insoluble protein providing elasticity to biological tissues such as lung, ligaments and blood vessels. The hydrophobic domains undergo a hydrophobic collapse upon crossing a lower critical solution temperature (LCST) , which can cause both compaction of individual chains, and association of chains into assemblies. Although key to the elasticity of elastin and stimuli-response of ELPs, a comprehensive mechanistic characterization of the static and dynamic aspects of the collapse is missing, which is linked to the difficulties in disentangling dynamical and structural aspects of assemblies, individual molecules and internal chain motions in this complex system. In particular, the dynamical state in the collapsed hydrophobic domains is highly debated (fluid-like structure versus specific stacking). In addition to their imminent physiological and biomedical relevance, ELPs are also models for intrinsically disordered proteins with a profound impact on cellular organization, but so far limited experimental opportunities to characterize structure and dynamics on a macromolecular scale.

Experimental results

(a) SANS. We measured samples of 50 mg/ml of protonated ELP and glycine-deuterared ELP (sequence: $GVG(VPGVG)_3$, where G = glycine, V = valine, P = proline) in phosphate buffer. Temperature-dependent SANS data were obtained on D11 using two configurations (1.7 and 16 m with collimations of 4 and 16.5 m). An example data set is shown in Fig. 1.

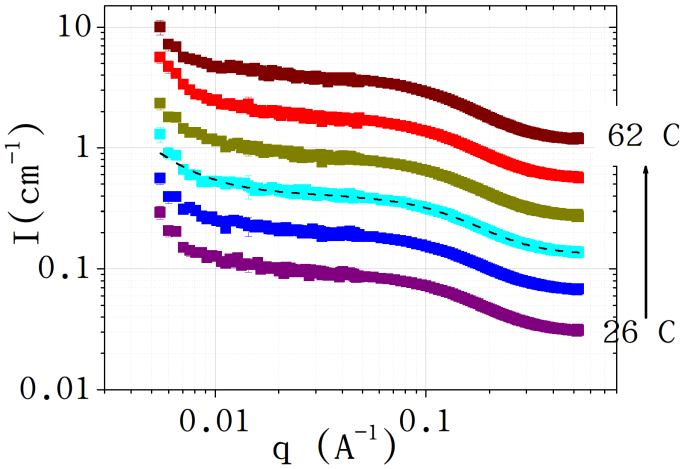

Figure 1: Temperature-dependent SANS data set on short hELP. The dashed line on the cyan curve represents a double-Flory fit based on a polymer equation inspired by Matt et al., 2019 (DOI: 10.1039/C9SM00583H).

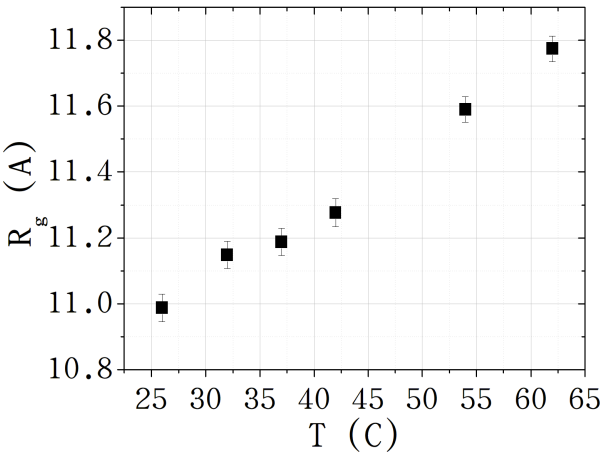

Figure 2: Radii of gyration determined from double-Flory fits to the SANS data.

From the fits to the data, radii of gyration were extracted. A slight increase is observed, which may indicate a certain degree of self-assembly of the short ELPs into larger structures.

(b) QENS. We measured the identical samples as in D11 for a range of temperatures from 28 to 67 °C. Data were sliced into an equidistant q array from 0.4 to 1.4 A^{-1}, normalized to the vanadium scattering and reduced for scattering of the sample cylinder and buffer background using standard scripts available at IN5. We obtain a good representation of the QENS signal with only one Lorentzian fit. Analyzing the relaxation rates, normalized for temperature and water viscosity, we obtain no strong change of flexibility during the entire temperature range, stressing that local chain flexibility is high even in the formed coacervates of ELPs at higher temperature. At the transition around 45 °C, we obtain interestingly a slightly enhanced dynamics.

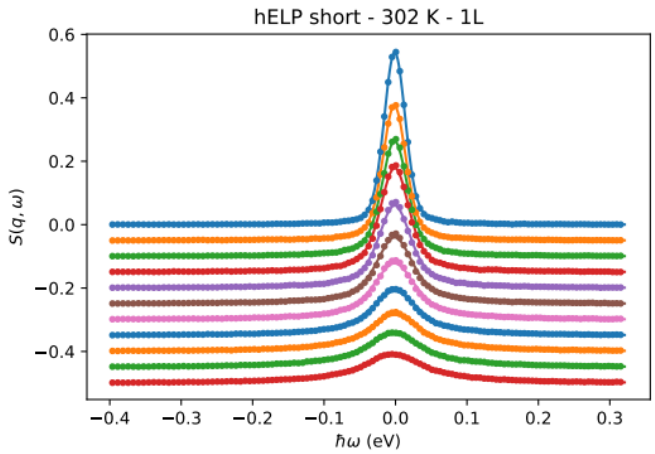

Fig.3: Dynamic structure factor of the ELPs at a temperature of 302 K (symbols) after normalization and background reduction. All data were fitted with a single Lorentzian shape (line).

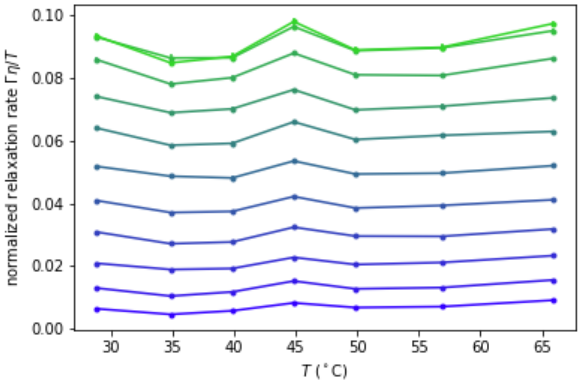

Fig.4: The normalized relaxation rate for low (blue) to high q (green) shows no strong variation over the entire temperature range, apart from a transient increase around the transition.

Conclusions. The combination of QENS and SANS allows us to show that these short peptide chains indeed have a tendency to form clusters with increasing temperature, which however is not reflected to a great extent in the local dynamics.