Experimental report

13/12/2017

Proposal: 9-13-663 **Council:** 4/2016

Title: Influence of soft confinment on the Inverse Temperature Transition ofan elastin-like peptide

Research area: Physics

This proposal is a new proposal

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Samples: D2O+GVG(VPGVG)3
D2O+AOT+Octane+GVG(VPGVG)3

Instrument	Requested days	Allocated days	From	To
D33	2	2	12/12/2016	14/12/2016

Abstract:

Elastin like peptides undergo an inverse temperature transition. So the peptide will fold upon heating. Simulations indicate that this transition leads to a significant change of the radius of gyration. With small angle neutron scattering this quantity is easily accessible and can be followed over a wide range of temperature. In the proposed experminet we intend to study this transition in bulk and in a soft confinement. The confinement is provided by a microemulsion in the droplet phase. By an appropriate deuteration scheme the scattering contrast will be mainly between the peptide and its background. Thus it will be possible to follow the inverse temperature transition, even in confinement.

Aim

Our aim was to investigate the structural implications of the inverse temperature transition (ITT) of GVG(VPGVG)$_3$ with the help of small angle neutron scattering (SANS). Previous studies [1–3] found that the GVG(VPGVG) peptide undergoes an ITT in the temperature range of 25°C - 70°C and simulations [4] suggest that the radius of gyration R_g of the peptide decreases by about 50% when folding due to the ITT. Further increasing the temperature would cause the peptide to unfold again and to denature. Another cause of denaturation is an unstable pH-environment, so the peptide was dissolved in a 10mM phosphate buffer to prevent denaturation. Further we wanted to combine our previous work on polyethyleneglycol contained in water droplets in an water-in-oil microemulsion [5] and the results of our peptide in bulk SANS measurements. To achieve this we prepared buffer-in-oil microemulsions containing the deuterated buffer, deuterated octane and deuterated AOT and peptide. The droplet volume fraction in all samples was 30% and the droplets have a radius of about 21 Å as confirmed by SAXS measurements. We prepared 5 samples with an average number Z of peptide chains per droplet of 0, 1, 2, 3 and 4, in order to not only study the influence of confinement on the ITT, but the influence of concentration inside a droplet as well.

Experimental details

The SANS measurements were performed on D33 with a wave length of $\lambda = 5$ Å and detector distances of 2.0 m, 5.0 m and 12.0 m yielding a q range of $0.010\,\text{Å}^{-1} \leq q \leq 0.566\,\text{Å}^{-1}$. The raw data was radially averaged, corrected for electronic background and empty cell scattering, and normalized to the scattering from water using standard ILL software.

For both experiments glass cuvettes were used as sample cells and the temperature was controlled to range from 25 °C to 70 °Cfor all samples.

Results

Peptide in buffer solution

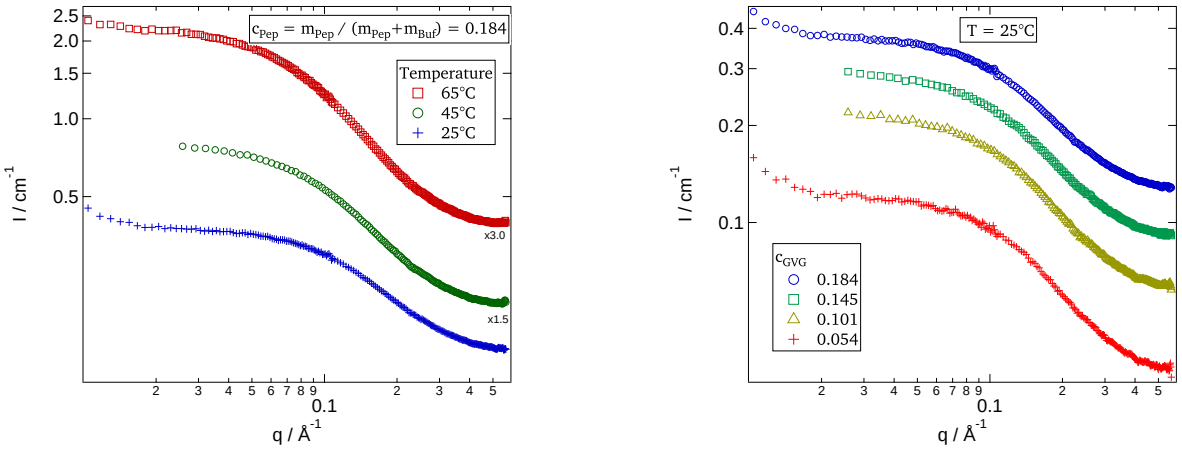

Figure 1: SANS data of the measured peptide in water: On the left is the temperature of the bulk sample with a mass fraction of 0.184 (The data is shifted). On the right is the data of all bulk samples at 25°C. The data is not shifted.

The results of the SANS experiment on the GVG(VPGVG)$_3$ in buffer solution are shown in figure 1. Since the conformation of the peptide chain is unknown, a model free Guinier-approach [6] is used to describe the data in the low q-range. This describes the data very well as can be seen in figure 2. Figure 2 also shows the radius of gyration R_g derived from the Guinier approach. R_g shows no sign of an ITT for the higher concentrations in the observed temperature range, but instead increases linearly with temperature. But for the lowest concentration there could be an indication of an ITT at about 45°C. Also the increase in R_g is much lower for the sample containing the least amount of peptide.

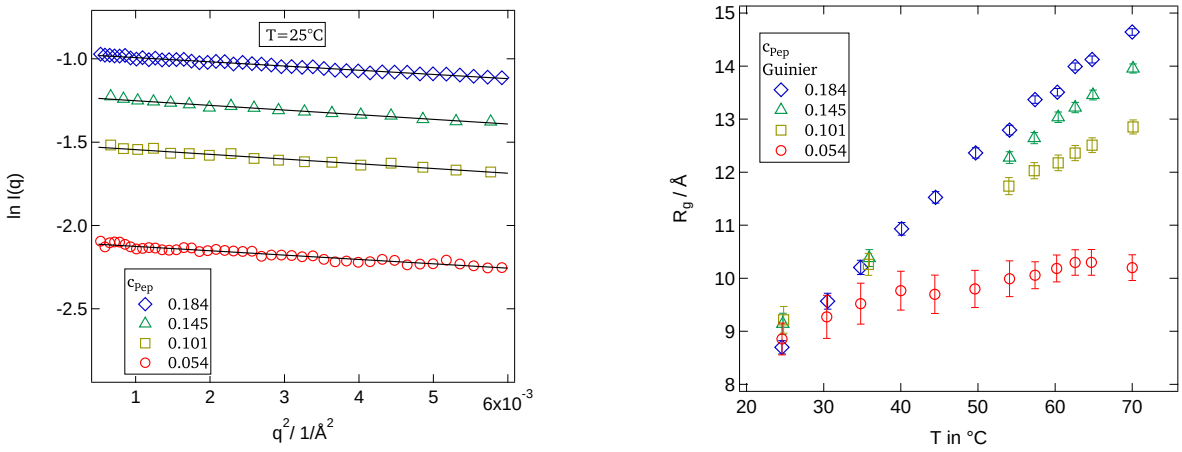

Figure 2: Left: Guinier representation with the linear fit. Right: The radius of gyration derived from the Guinier approach.

In order to further investigate, if the peptide underwent an ITT/change in structure, one can look at the Kratky-plot [7] in figure 3. There it can be seen that the sample with the lowest concentration of peptide does not change its structure when heated from 25 °C to 70 °C. It should be also mentioned that in the Kratky-plot the peptide shows the behaviour of a flexible chain instead of a folded particle. The mass of the scattering particle can be calculated by using the integral $\int I(q) \cdot qdq$ [8]. The calculated mass of the scattering particle is still under investigation. Still the increase in R_g and the unchanging conformation suggested by the Kratky-plot, could indicate a clustering of peptide chains. This assumption could be supported by an increasing mass of the scattering particle.

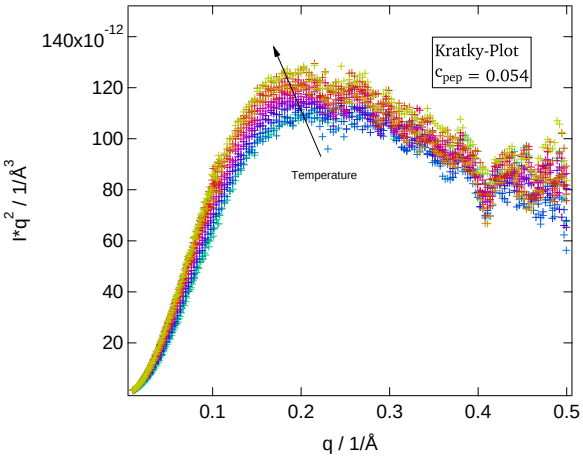

Figure 3: Kratky-plot for the sample containing the lowest concentration of peptide at all observed temperatures. The arrow indicates the direction of temperature increase.

Peptide in microemulsion

The results of the measurements of the microemulsions samples are shown in figure 4

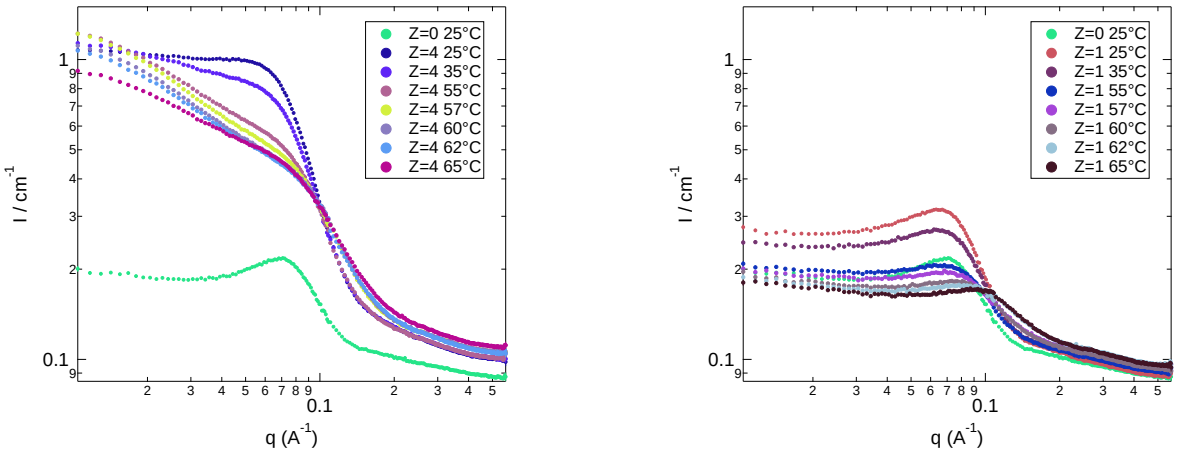

Figure 4: Left: Results for the microemulsion containing Z=4 peptide chains per droplet. Right: Results for the microemulsion containing Z=1 peptide chains per droplet. Both plots also show the result for a microemulsion containing no peptide.

One can see that adding peptide chains into the droplet increases the intensity. It can also bee seen that there is a crossover at around $q = 0.1\,\text{Å}^{-1}$ where the intensity of the lower temperature measurements drops beneath the intensity for higher temperature measurements. For larger Z values at higher temperatures there is a sign of clustering at low q-values denoted by the power law of the data. It should also be noted that the empty microemulsion shows a scattering signal similar to the data of the microemulsion containing one peptide chain. This feature seems to be caused by the droplet, rather then the peptide contained in inside of it. Due to the high volumefraction of 30% of the droplets, the percolation temperature of the system should be rather low. So the droplets could form transient clusters, which would cause the observed intensity. This could also be the reason why the intensity for the high temperature measurements for $Z = 1$ falls below the intensity of the empty microemulsion at low q-values (see figure 4 right panel). These measurements are still being evaluated.

References

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