



Universiteit  
Leiden  
The Netherlands

## Probing spatial heterogeneity in supercooled glycerol and temporal heterogeneity with single-molecule FRET in polyprolines

Xia, T.

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## 5 Small-angle neutron scattering on supercooled glycerol

**Abstract** – We investigate aging of supercooled glycerol by means of small-angle neutron scattering. Two samples are prepared in cuvette cells with thermal histories similar to the previously established one (Chapter 2). We observe the growth of solid-like structures in one sample only, evidenced by both direct visual inspection and the scattering spectra. A new peak appeared around  $0.1 \text{ \AA}^{-1}$  after annealing at 240 K for hours. It is a hallmark of this solid-like state, for it is clearly absent from the pure liquid spectrum of glycerol at the same temperature and from the crystal spectrum.

## 5.1 Introduction

Understanding supercooled liquids and their glass transition is still an open problem in material science. Early concepts for the explanation of the liquid to glass transition postulate the existence of cooperatively rearranging regions (CRR), which are believed to be responsible for the observed spatial and dynamical heterogeneities in many glass-forming systems [4]. Although heterogeneity is readily detected experimentally, its characteristic length scales still remain unclear. The scale of a few nanometers was found by NMR [7, 8] and dielectric relaxation spectroscopy [9, 10]. However, light and X-ray scattering experiments have detected correlation lengths on the order of 100 nm [11, 12]. Glycerol, an archetypal glass-former, has been investigated extensively by many different methods [9, 10, 57, 87–89], but nonergodic behavior in the supercooled regime was never reported until in a recent single-molecule study of local relaxation of supercooled glycerol. This study revealed an extremely long-lived heterogeneity at temperatures well above its glass transition [1]. The associated time scale is on the order of days, about one million times longer than the alpha-relaxation time. Later complementary rheological studies of supercooled glycerol also uncovered a novel viscoelastic behavior at temperatures above  $T_g$  (Chapter 2), consistent with the single-molecule observation that some solid-like structures already developed above the glass transition. To explore the structural origin of this solid-like state, we perform small-angle neutron scattering (SANS) experiments on supercooled glycerol. This technique has proved to be a powerful tool in materials science, which can provide structural information on length scales of 1–100 nm. The technique of ultra small-angle neutron scattering (USANS) further raises the upper resolution limit for structural studies by two orders of magnitude (up to 50  $\mu\text{m}$ ) [100, 101] and hence overlaps with light scattering and microscopy. Similar to the often complementary techniques of small-angle X-ray scattering (SAXS) and light scattering, SANS is very useful because many materials, substances and biological systems possess interesting and complex features in their structure, which match the length ranges that the technique can access. On the other hand, unlike X-ray photons, which interact with the electron cloud surrounding the nucleus, neutrons interact with atomic nuclei and because they are electrically neutral particles, they are deeply penetrating, and are therefore more able to probe bulk materials. As a consequence, SANS enables the use of a wide range of sample environments that are sometimes difficult to use with synchrotron X-ray sources. Another advantage of neutrons over X-rays is that for neutrons the scattering cross section does not vary with the number of nuclei as for X-rays, but is completely different from an element to another.

For instance, H, C, N and O have non negligible scattering cross sections for neutrons, which is not the case with X-rays.

In neutron scattering, the scattering cross-section for the nucleus is the sum of two terms,

$$\sigma = \sigma_c + \sigma_{inc},$$

where  $\sigma_c$  is the cross-section for coherent scattering and  $\sigma_{inc}$  for incoherent scattering. It is the former one that can produce interference and determine the intensities of the Bragg reflections of crystals for neutrons. The latter one in contrast only leads to an isotropic (flat) background. Hydrogen, for instance, has a very large total scattering cross-section ( $\sigma_H = 81.5 \times 10^{-24} \text{ cm}^2$ ) among elements and isotopes. However, the coherent contribution ( $\sigma_{Hc}$ ) is only  $1.8 \times 10^{-24} \text{ cm}^2$ , less than 3% of the total scattering cross-section, which means that the signal from coherent scattering will almost completely drown in the background of incoherent scattering when performing neutron scattering on hydrogenated samples. This, however, is not the case for deuterium, because its total scattering cross-section ( $\sigma_D = 7.4 \times 10^{-24} \text{ cm}^2$ ) has a large coherent component ( $\sigma_{Dc} = 5.4 \times 10^{-24} \text{ cm}^2$ ).

The remarkable difference in scattering contrast between hydrogen and deuterium makes deuterium-labeling techniques practically very useful since partial substitution of deuterium for hydrogen in molecules can increase the signal from the coherent scattering, thus competing with the isotropic background due to the incoherent scattering from hydrogens. This, for example, has been used in polymer studies to specifically “stain” certain polymer chains by replacing the relevant hydrogens with deuteriums during the synthesis and make them “visible” in concentrated solutions and the condensed states [102]. Such selectivity or the enhanced contrast could not be achieved without working in dilute solutions by light and small-angle X-ray scattering. This unique feature of SANS also makes it particularly useful for the biological sciences [103, 104], since biological molecules are usually dissolved in water, and hydrogens which are loosely associated with these molecules are able to exchange with any deuteriums in the solvent. This usually has minimal functional effect on the sample but has dramatic effects on the scattering.

Here we use fully deuterated glycerol samples for SANS measurements for the reason mentioned above. We apply thermal treatments, similar to the previously established one (Chapter 2), to two glycerol samples and the scattering signal is monitored during annealing at 240 K. In one sample, we observe the growth of the solid-like structures at 240 K and the scattering spectrum shows a distinct peak appearing around  $0.1 \text{ \AA}^{-1}$ , which is not seen in the pure liquid

state of glycerol nor in the crystal.

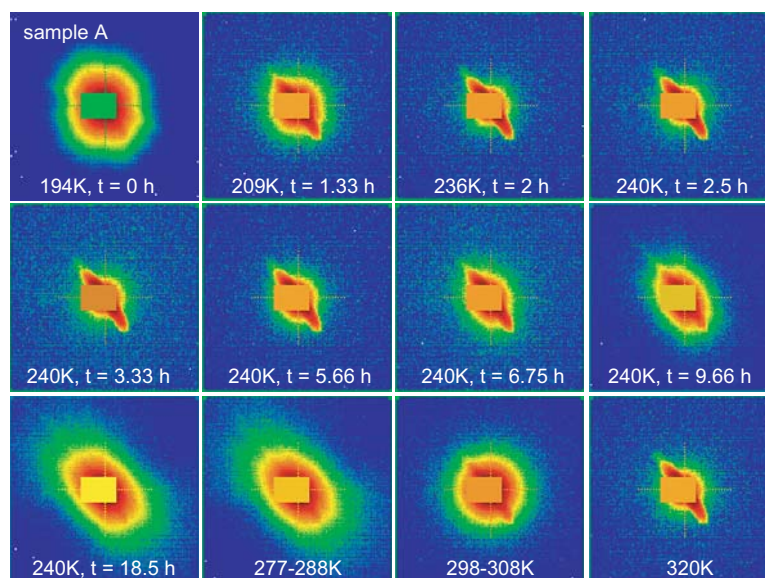
## 5.2 Experimental methods

The small-angle neutron scattering experiment was performed on instrument D11 at the Institut Laue-Langevin (ILL) in Grenoble, France. A neutron beam with a wavelength of 6 Å was used for the scattering measurements. The small and large angle range was chosen to cover the  $Q$  range from 0.008 to 0.431 Å<sup>-1</sup> by changing the detector distance. The deuterated glycerol (DLM-558-5, Cambridge Isotope Laboratories, Inc) was loaded into two quartz cells (100 QS-2 mm, Hellma). These two samples have been measured. The first one (sample A) was prepared in a cryostat with a slow cooling (5 K/h) from 300 K to 194 K followed by a long waiting time (about 43 hours) at a fixed temperature (194 K). During the installation of the cryostat on D11, the temperature regulation was off for about one hour and the sample temperature dropped down to 113 K. The neutron scattering measurements were started when the sample temperature was stabilized again at 194 K. The sample was then heated up to 240 K with a rate of 60 K/h and kept at this temperature for about 16 hours. Due to an unexpected technical problem on the data acquisition, the data during the second half of the aging at 240 K were not saved. We therefore only measured the beginning and the end of the last part of aging. After that, we further heated up the sample to 328 K (30 K/h) to record the spectrum of the normal liquid state and then applied a fast cooling (60 K/h, from 328 K to 240 K) to the sample to obtain the signal arising from the usual supercooled liquid state of glycerol, as a control.

The second sample (sample B) was prepared in a different cryostat with the same slow cooling profile, but it was kept at 194 K for 4 hours before transfer into the D11-cryostat. It is clear that this transfer procedure is not satisfying, due to sudden temperature changes during transfer into liquid nitrogen of a fragile sample. The annealing at 244 K was then monitored for 16 hours.

A deuterated polycrystalline glycerol sample prepared in a quartz cell (100 QS-1 mm, Hellma) was also measured at 243 K as an additional control.

The raw 2D data were treated with a standard procedure in Large Array Manipulation Program developed at the ILL. Because the raw 2D data show strong anisotropy in the scattering signal (see Figure 5.1), which is partly attributed to the reflection from the cryostat, additional care has been taken for the data treatment.



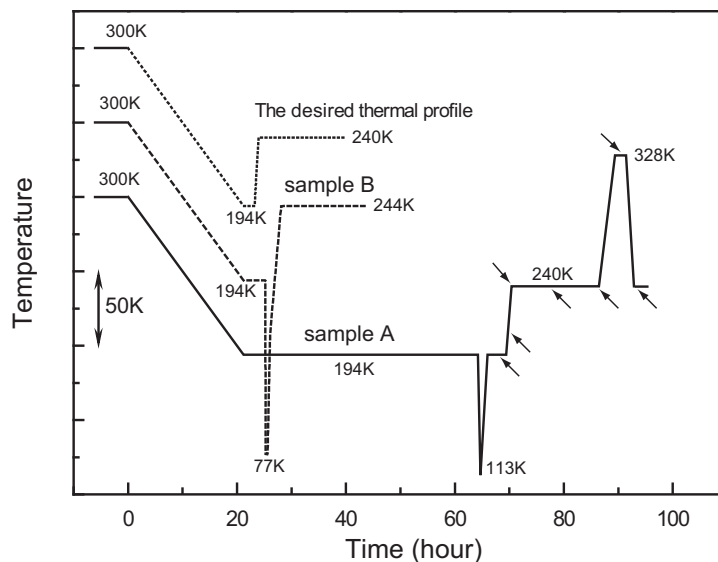
**Figure 5.1:** The raw 2D scattering data of sample A as a function of temperature measured in the small angle range. The diagonal elongated spot (for example on the third image at 236 K) is partly due to a parasitic reflection from the cryostat. The one dimensional scattering spectrum was obtained by radial averaging of the reduced raw 2D data. The rectangle shade in each image indicates the region in which the data have not been taken for radial averaging.

### 5.3 Results and discussion

The work presented in Chapter 3 has shown that the behavior of glycerol has a strong thermal history dependence and an initial slow cooling period appears to be crucial for the development of the solid-like structure. We therefore applied this slow cooling procedure to the two samples measured in this work. Figure 5.2 shows their actual thermal histories and they both, to different extents, deviate from the desired thermal profile because of practical reasons (see Experimental methods section). Nevertheless, we observed the solid-like structures developed in sample A after annealing at 240 K for about 8 hours, which was even visible by eye (data not shown). The whitish material started to grow from one side of the wall in the cuvette cell and it expanded with time. The raw 2D data of this sample also showed a steady increase in the radius of the scattering signal during aging at 240K (see the images at 240K in Figure 5.1). The 1D scattering spectra of this sample are plotted in Fig-

ure 5.3. Figure 5.3 A shows the control spectra of the pure liquid state of glycerol (sample A) at 240 K ( $T_g + 50$  K) obtained by a fast cooling (60 K/h, from 300 to 240 K) into the supercooled regime and at 328 K ( $T_m + 36$  K) in the normal liquid regime, in comparison with the spectrum of the crystal sample recorded at 243 K. Upon heating the sample to 240 K after a long period of quenching at 194 K, the small angle scattering signal between  $0.008 \text{ \AA}^{-1}$  and  $0.07 \text{ \AA}^{-1}$  first decreased dramatically with increasing temperature, whereas the signal in the large angle range remained unchanged (see Figure 5.3 B), suggesting a pronounced rearranging process taking place mainly on the large scales (9-63 nm). However, after annealing at 240 K for a few hours the signal started to recover, indicating that a restructuring can occur after incubating at a higher temperature. Meanwhile, in the large angle range, a new peak appeared around  $0.1 \text{ \AA}^{-1}$  during this annealing process (see Figure 5.3 C), corresponding to a characteristic length scale of 6 nm approximately. The  $0.1 \text{ \AA}^{-1}$  peak is a hallmark of the solid-like state of glycerol, which is clearly absent in the pure liquid state obtained by a fast cooling (see the inset in Figure 5.3 C). This peak is also absent from the crystal sample. Upon further heating the solidified glycerol to temperatures below 292 K, the shape of the spectra did not change much except for a slight increase of the overall signal (data not shown). When the temperature crossed the melting point of the crystalline glycerol, the scattering signal dropped and the peak disappeared (see the molten spectrum in Figure 5.3 A), indicating the melting of the solid-like structures.

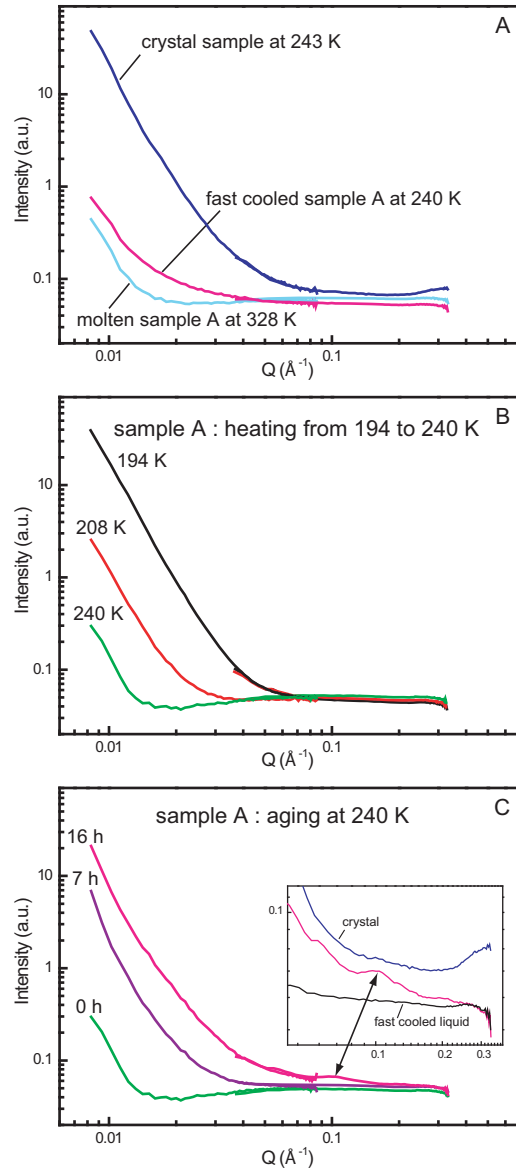
Sample B, however, was transparent all the time at 240 K and the spectra revealed no changes at this temperature for 16 hours (see Figure 5.4). It is not clear why the solidification did not happen to this sample. However, it should be noted that the thermal histories of the two samples are not the same. For example, sample A had a period of 43 hours at 194 K, about 10 times longer than that of sample B (about 4 hours). Sample B experienced a much lower temperature (77 K) than sample A and was annealed at 244 K, instead of 240 K. All these differences may contribute to the failure of partial solidification in sample B. A similar problem was also seen in the rheological measurements of glycerol in the plate-plate geometry (in Chapter 3) where only one out of six runs showed the solid-like behavior, even though the thermal history was well controlled. It seems that solidification of supercooled glycerol has a random nature and its onset is influenced by many external parameters. Some of them are controllable in practice, such as the thermal history and the sample geometry. Some are difficult to control, like the purity of the sample and the properties of the interface between the sample and the measurement device. This somehow coincides with a general scenario of crystallization of



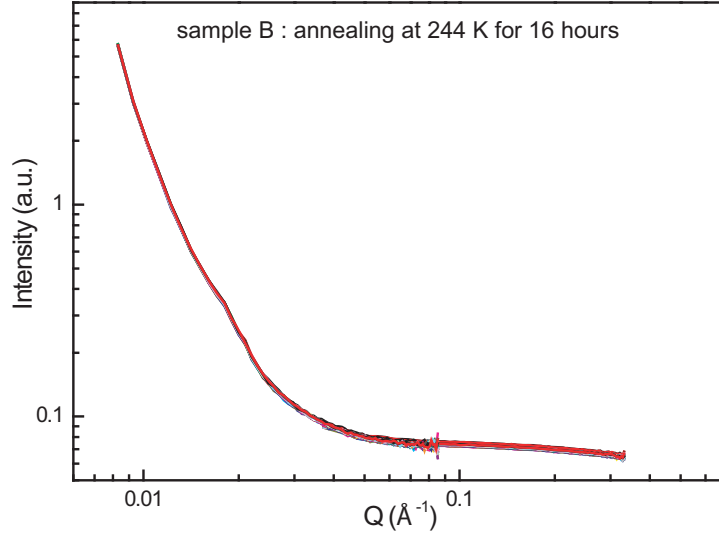
**Figure 5.2:** The thermal histories of the two samples in comparison with the established thermal profile. The lines are vertically shifted for clarity. The dotted line is the desired thermal profile, which we would like to reproduce. The solid and dashed lines are the actual thermal profiles for sample A and sample B, respectively. The initial slow cooling rate is about 5 K/h. The sudden drops of temperature far below the glass transition (190 K) in sample A and B are due to the practical reasons (see Experimental Methods). The arrows indicate when the spectra of sample A in Figure 5.3 were taken.

melts where the number of nuclei in the melt, crucial to the final crystallization, is greatly influenced by the size and history of the sample and the condition of the container. The effect of amount of materials on the frequency of nucleation has been investigated by many people. Tammann, for instance, noted that 6 g of phenol required, on the average, 2810 s to crystallize, whereas 30 g took only 33 s under the same conditions [105]. The stochastic nature of crystal nucleation can also be seen in crystallization of polymers in a confined geometry [106].

Is solidification of supercooled glycerol related to a crystallization process? Based on the preliminary results from sample A, this is still an open question since the spectra of the solidified glycerol are different from that of the crystal sample. In addition, the Q range used in this work is outside the range where



**Figure 5.3:** The scattering spectra of sample A at different stages. (A) Pure liquid state of sample A at 240 K (by a fast cooling) and 328 K, in comparison with a crystalline state of a control sample. (B) Heating from 194 to 240 K. (C) Aging at 240 K. The inset is the enlarged view of the region in which a new peak centered around  $0.1 \text{ \AA}^{-1}$  was clearly identified after 16 hours at 240K, which was not seen in the liquid and crystalline states of glycerol in this temperature range.



**Figure 5.4:** 24 spectra of sample B measured every 40 min at 244 K for a period of 16 hours. They are all perfectly overlapped

the Bragg peaks are normally seen, i.e., between  $0.5 \text{ \AA}^{-1}$  and  $5 \text{ \AA}^{-1}$ . Nevertheless, the steady increase of the scattering signal around  $0.1 \text{ \AA}^{-1}$ , leading to the formation of a well defined peak, during the aging at 240 K is a unique feature for the solid-like state of supercooled glycerol. Schwickert et al. have recently performed a similar study on supercooled triphenyl phosphite (TPP) [107], a molecular liquid that shows an anomalous solid-like behavior at temperatures above its glass transition [80, 81]. They characterized the early stages of cluster growth in supercooled TPP by using small-angle neutron scattering and they found a pronounced increase of the scattering signal around  $0.07 \text{ \AA}^{-1}$  during annealing in the temperature range of 210 K to 214 K, which is very similar to what we observed from the solidified glycerol here. The solid-like state of glycerol, therefore, seems to be closely related to the solid-like state of TPP, also known as the glacial state [80, 81]. The structural description of this glacial state is still under debate. The experiments performed at higher temperatures (216 K-235 K) indicate that the glacial state of TPP is a mixture of micro-crystallites and non-transformed supercooled liquid [84], whereas the measurements at a lower temperature (213 K) suggest that the glacial state is a homogeneous glassy state of a denser liquid free from micro-crystallites [82, 83].

The sample (thermal) history is therefore an important experimental parameter, which should be carefully taken into account before any interpretation of own results or comparison with others' is made, especially in the study of viscous molecular liquids whose behaviors have a strong thermal history dependence.

## 5.4 Conclusion

We studied the aging of supercooled glycerol by small-angle neutron scattering. Two samples were measured and we only observed the solid-like structures in one sample where a new peak formed around  $0.1 \text{ \AA}^{-1}$  in the scattering spectrum after aging at 240 K for hours. This peak was absent in both the liquid and crystalline states of glycerol at the same temperature. Because the Q range chosen in this work does not cover the range in which the Bragg peaks normally appear, we do not know whether the solid-like structures contain crystals or not. Future studies in the large Q range at a lower temperature, for example at 220 K or even 205 K, combined with different cooling rates shall give more insight into the structural origin of this solid-like state in supercooled glycerol.

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