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

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### 3 Aging and solidification of supercooled glycerol

**Abstract** –We experimentally investigate the solidification of supercooled glycerol during aging that has been observed in Chapter 2. We find that a slow cooling at 5 K/h prior to the aging is required for solidification to take place. Furthermore we show that the time of onset depends strongly on the aging temperature which we varied between 220 K and 240 K. The nature of the solid phase remains unclear. The experiments show that upon heating the solid glycerol melts at the crystal melting point. However, rheology experiments in the plate-plate geometry revealed the growth of a soft, slush-like phase that is distinct from a crystal grown by seeding at the same aging temperature. The slush-like glycerol grows from a nucleation point at almost the same speed as a seeded crystal quenched to the same temperature, but its shear modulus is almost two orders of magnitude smaller than the crystal phase, which we measure independently. While solidification was reproducible in the Couette geometry, it was not in the plate-plate geometry.

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### 3.1 Introduction

Recent single-molecule optical experiments on supercooled glycerol near the glass transition have shown evidence for dynamical heterogeneities on the molecular level [1]. Most strikingly, the observed lifetime of the spatial heterogeneity is on the order of days, about six orders of magnitude longer than the typical time for a glycerol molecule to reorient. Moreover, as was shown in Chapter 2 [74], the rheology of supercooled glycerol near the glass transition exhibits a novel viscoelastic behavior after sufficient aging of the sample. This was a surprising finding, given that supercooled liquids are thought to be pure Newtonian liquids whose viscosity diverges at the glass transition temperature  $T_g$ . This non-Newtonian behavior has been conjectured to be a consequence of these microscopic heterogeneities [1].

In this chapter we expand on the work presented in Chapter 2 where our experimental setup was restricted to steady shear measurements which can cause “breaking events” that rejuvenate the sample. Moreover, we did not have optical access to the sample as it aged. In this work we address these shortcomings by performing the experiments in a commercial rheometer.

We employ an Anton Paar MCR 501 rheometer with an environmental chamber that enables us to probe the supercooled glycerol with small strain amplitude oscillatory measurements to avoid the previously observed “breaking events” of the fragile network while measuring its response. We perform experiments first in a Couette geometry with similar dimensions as in Chapter 2. We monitor the linear response of supercooled glycerol as a function of aging time at different aging temperatures above the glass transition. We find that an initial slow cooling period is crucial for the formation of the solid-like state. However, the deformation of the measurement tools (apparatus compliance) may limit the maximum measurable rigidity of the sample [75]. We could not ascertain whether the solid-like structure we measure in the Couette geometry is a crystal growing in a liquid or a solid-like phase distinct from the normal crystal.

We therefore repeated the experiment in a plate-plate geometry, where gap and plate size were chosen such that the tool compliance is negligible. In addition the sample can be optically accessed in this geometry. Surprisingly, we could not reliably reproduce the solid-like state in the plate-plate geometry, although we applied the same temperature history as in the Couette geometry. Nevertheless, we found evidence of a slush-like phase that grows from a nucleation point at the speed of the crystal phase, but has a soft slushy consistency with a shear modulus about two orders of magnitude lower than the crystal phase. The storage modulus of the slushy phase is within an order of magni-

tude of the modulus found in Chapter 2, although the aging temperatures were different (240 K versus 205 K). When slowly heated, the slush-like phase eventually melts at the crystal melting point of glycerol, which indicates that the slush either contains crystallites or turns into a crystal at higher temperatures.

## 3.2 Experimental methods

In order to probe the solidification of glycerol during aging we employed a Anton Paar MCR 501 rheometer with two measurement geometries. The first is a Couette geometry (Anton Paar CC 20) similar in dimensions to that used in Chapter 2. The gap size between inner and outer cylinder is 1 mm. The inner cylinder has a radius of 10 mm and is 30.1 mm long, with a conical bottom. The compliance of the tool and the cup as stated by the manufacturer is 1.65 mrad/Nm and 4.5 mrad/Nm, respectively. The total sample volume is about 7 ml.

Our customized plate-plate geometry (see Figure 3.6A) consists of two parallel discs with an upper disc radius of 1.5 mm and gap sizes between 2.0 and 2.3 mm. The small radius and large gap size ensure that compliance effects are negligible, which we checked explicitly (see Figure 3.5). Another advantage of this geometry is the optical access through the observation window of the chamber. We mounted a camera with a microscope lens outside the chamber which was focused on the sample. The sample was backlit with a LED behind a diffusing plate inside the chamber; the LED was turned on all the time and had no heating effect on the sample. The disadvantage of the geometry is the lower accuracy because of increased edge effects - the sample between the plates is not cylindrical but has a meniscus - and the lower sensitivity. It can only be used for samples with high moduli. Also, the sample volume, about 0.014 ml, is much smaller than in the Couette cell.

Both geometries are placed in a low temperature environmental chamber (Anton Paar CTD 450), which allows cooling down to 173 K. The cooling is provided by a liquid nitrogen evaporation unit (Anton Paar EVU), that coupled with the heating elements in the chamber ensures constant temperature environment with fluctuations of around 0.1 K. The flooding of the chamber with nitrogen vapor also prevents water uptake of the glycerol sample. Due to the high consumption of liquid nitrogen (about 6 l/h), we had to change nitrogen vessels every 15 – 20 hours. Furthermore, we restricted our measurements to aging temperatures of 220 K and above, which is in contrast to our studies in Chapter 2 where the aging temperature was 205 K and the waiting time extended to weeks.

A temperature probe (Anton Paar Pt 100) is located at the bottom of both the Couette and plate-plate geometry and allows the rheometer to control the temperature in the chamber (the temperature difference from the center of chamber to the wall can be up to 4 K). A more accurate, better calibrated silicon diode (Lakeshore, DT-670-CU-1.4L) is attached directly to the cup of the Couette cell and the bottom plate of the plate-plate geometry, and was used to measure the absolute temperature.

During the course of the experiment we used two ultrapure samples (99.5% purity) of glycerol from two different suppliers, Invitrogen and Fluka. The bottle of glycerol was sealed with Parafilm and kept in a desiccator. Both samples could be solidified in the Couette geometry.

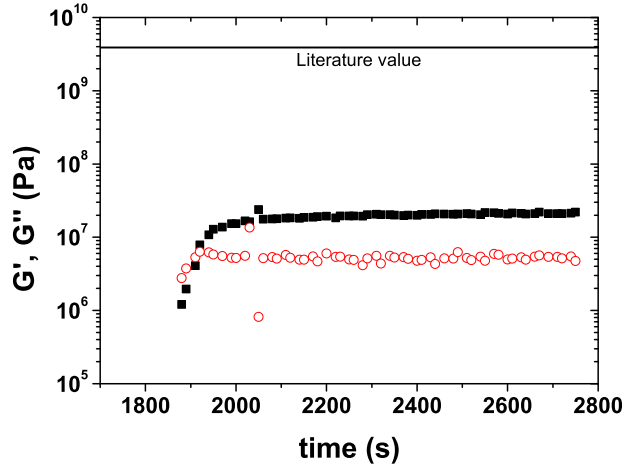
## 3.3 Results

### 3.3.1 Viscosity measurements

First we measured the viscosity of glycerol as a function of temperature in a Couette cell and a plate-plate geometry, respectively, to test the accuracy of these two different geometries. In both geometries we did strain rate-controlled measurements. It is important to be cautious: when the elastic constant of the sample becomes comparable to that of the measurement cell, the compliance of the measurement device may overwhelm the deformations in the sample [75]. As is shown in Figure 3.1, the measured storage modulus of frozen water with the Couette cell plateaus around  $10^7$  Pa, which is two orders of magnitude below the actual shear modulus of ice (3.9 GPa) [76]. Although this compliance effect is expected in the Couette geometry, it does not affect the viscosity measurements at steady shear [75]. At each data point we waited 10 minutes before the measurement was taken to allow thermal equilibration of the sample. As can be seen in Figure 3.2, the measured values of the viscosity versus temperature are in good agreement with previous measurements by Schröter et al. [57]. In case of the plate-plate geometry the viscosity values are slightly lower, which can be attributed to lower accuracy due to edge effects (see Experimental methods section).

### 3.3.2 Solidification and importance of cooling rate

An initial slow cooling is important for glycerol to develop a solid-like structure. In order to create the solid-like state observed in Chapter 2, we first performed the aging experiments in the Couette Cell. We found that rapidly cooling down the sample to  $T = 192$  K, which is close to the glass transition

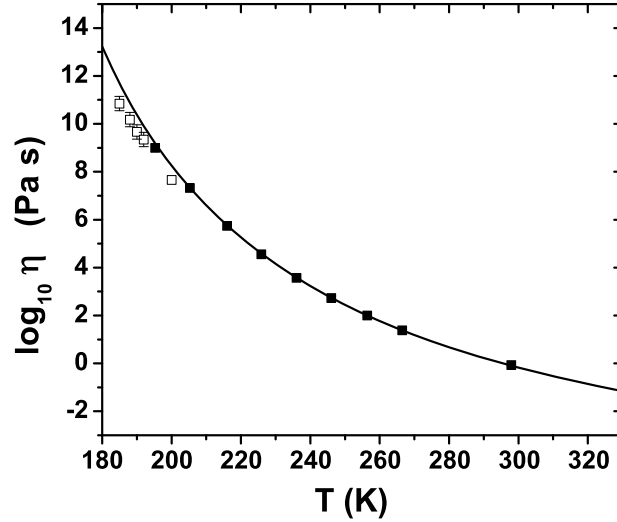


**Figure 3.1:** Measurement artefact in the Couette cell. Oscillation measurement ( $G'$ (■),  $G''$ (○)) of ice in the Couette cell at  $T = 268$  K. The oscillation test yields a storage modulus ( $G'$ ) that is two orders of magnitude lower than the actual shear modulus of ice due to tool compliance, indicating that the upper limit of the measurable shear modulus with the Couette cell is on the order of  $10^7$  Pa.

temperature  $T_g = 190$  K, followed by an aging at a higher temperature, irrespective of the aging temperature, did not lead to solidification reliably. The aging has to be preceded by a slow cooling period at a rate of 5 K/h.

The temperature protocol that leads to reproducible solidification is as follows (see Figure 3.3 A): first, the sample is cooled down to 260 K from room-temperature within about 20 minutes during which the temperature inside the environmental chamber is equilibrated. Then the sample is *slowly* cooled down further at a rate of 5 K/h to 192 K, which is just above  $T_g$ . After 2–3 hours at 192 K, the sample is warmed up to the desired aging temperature  $T_a$  at a rate of 60 K/h. When the  $T_a$  is reached the linear response is probed by oscillation measurements at  $f = 0.1$  Hz and a low strain amplitude  $\gamma = 5.3 \cdot 10^{-4}$ . The low strain amplitude was chosen to disturb the sample as little as possible.

In Figure 3.3 B-D we show the storage and loss modulus as a function of time for the glycerol in the Couette geometry at three different aging temperatures. In all cases, the development of a solid-like structure is evidenced by a steep increase of  $G'$  (and  $G''$ ) at time  $t_s$ . This increase is only observable several hours after the final aging temperature  $T_a$  has been reached. The solidification time  $t_s$  increases with decreasing  $T_a$ . The curves of the moduli as a function of time are not smooth and frequently exhibit kinks, i.e., sudden increases of

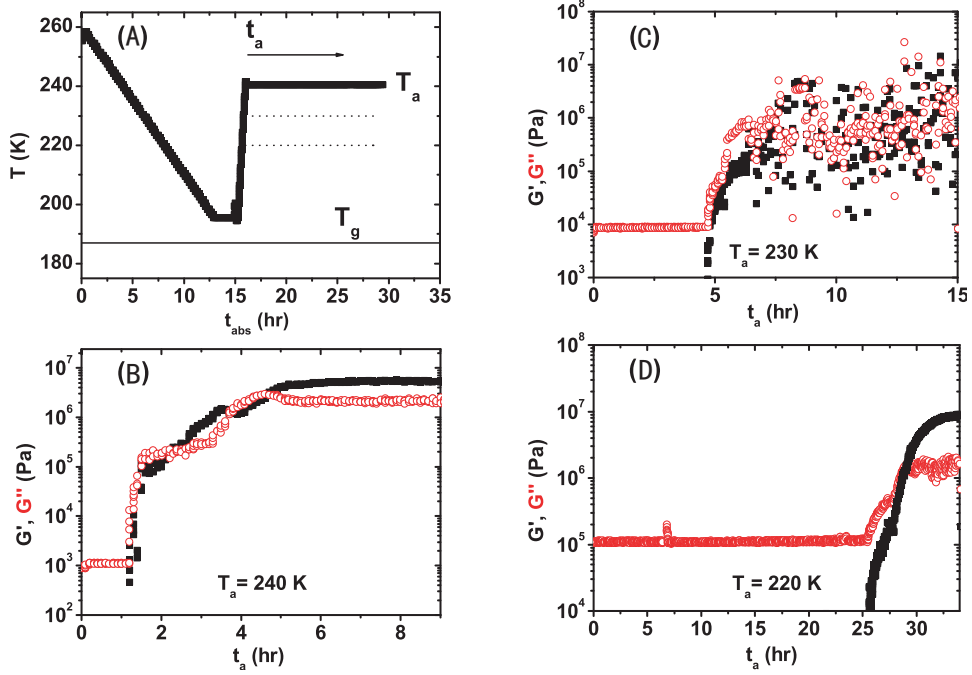


**Figure 3.2:** Dynamic viscosity versus temperature measurements of glycerol. All the measurements are obtained at steady shear (strain rate controlled) on the quickly cooled liquid state of glycerol. The solid symbols (■) are taken in the Couette cell, empty symbols (□) are measurements from the plate-plate geometry. The solid line is the VFTH (Vogel- Fulcher-Tammann-Hesse) fit from Schröter et al. [57]:  $\log_{10}(\eta/Pa \cdot s) = -7.1 + 1260/(T - 118)$ .

the time derivatives of the storage and/or loss moduli. These kinks might indicate meeting or merging events of some macroscopic solid-like clusters as they grow. Since the Couette cell is not transparent, we cannot test this.

Once the solid-like state is formed, it can be molten by heating the sample considerably. Figure 3.4 shows the magnitude of the complex viscosity of solidified glycerol obtained from the aforementioned procedure as a function of temperature. By warming up the sample at a rate of 12 K/h, we first observed a slight increase in the complex viscosity between 220 K and 288 K then a drastic drop when the temperature crossed 291.75 K, the melting temperature of the crystalline glycerol [77]. After that, the values of viscosity followed the literature values of supercooled glycerol. When the solid-like structure has completely molten, the sample was cooled down again from 300 K at a rate of 0.2 K/min and it behaved like a normal supercooled liquid.

The apparent shear modulus of the solid-like state, as shown in Figure 3.3, is two orders of magnitude lower than the modulus of crystalline glycerol, which is about 3.4 GPa, as derived from the transverse sound velocity in the



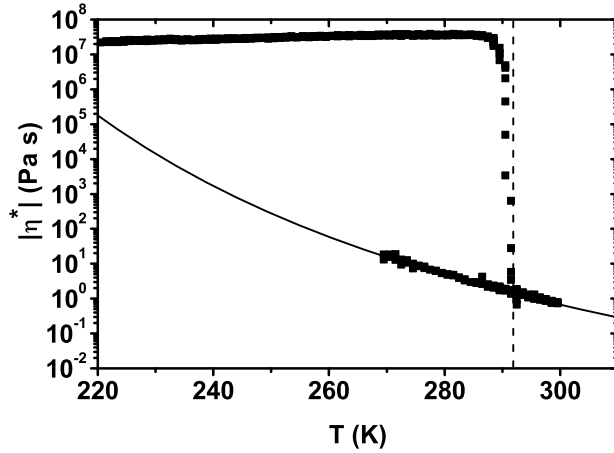
**Figure 3.3:** Aging and solidification of supercooled glycerol. (A) This is the temperature protocol used to create the solid-like state shown in panels (B)-(D). The sample is slowly cooled at 5 K/h from 260 K down to 192 K where it remains for 2 – 3 hours. It is then warmed up at 60 K/h to the desired aging temperature  $T_a$ . Panels (B)-(D) show the linear response,  $G'$  (■) and  $G''$  (○), of the oscillatory measurements during aging at  $f = 0.1$  Hz and a low strain amplitude  $\gamma = 5.3 \cdot 10^{-4}$  at different aging temperatures  $T_a$ : (B)  $T_a = 220$  K, (C)  $T_a = 230$  K and (D)  $T_a = 240$  K. Note: the bump in  $G''$  at  $t_a = 7$  h is due to the changing of the nitrogen vessel.

crystal [59]. Since the control measurements on frozen water have pointed out the compliance issue, the plateau values of the moduli of the aged glycerol in the Couette geometry cannot be trusted.

### 3.3.3 Measurements in the plate-plate geometry

In order to avoid complications due to the compliance of the tool we turned our attention to the plate-plate geometry with a relative wide gap and a small radius [75]. To ensure that the geometry is free of compliance artifacts we measured the  $\alpha$ -relaxation peak of the loss modulus in supercooled glycerol at different temperatures. After a sufficient equilibration period of the sample at



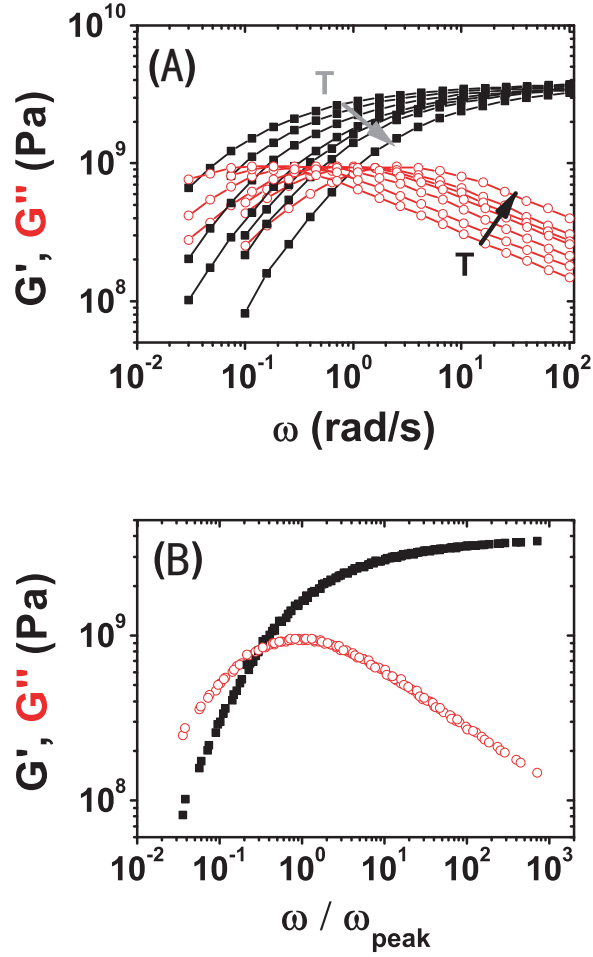


**Figure 3.4:** Modulus of complex viscosity monitored during melting and subsequent cooling. This is the same experimental run as shown in Figure 3.2 D. After aging at 220 K, during which the glycerol has solidified, we slowly heated the sample at 15 K/h while monitoring the linear response. At the melting point of the crystal phase of glycerol  $T_m = 291.75$  K [77], the modulus of the complex viscosity drops down to the values for the dynamic viscosity expected for liquid glycerol. Subsequent cooling from 300 K shows that the Newtonian liquid behavior is restored. The solid line is the same VFHT fit as in Figure 3.2.

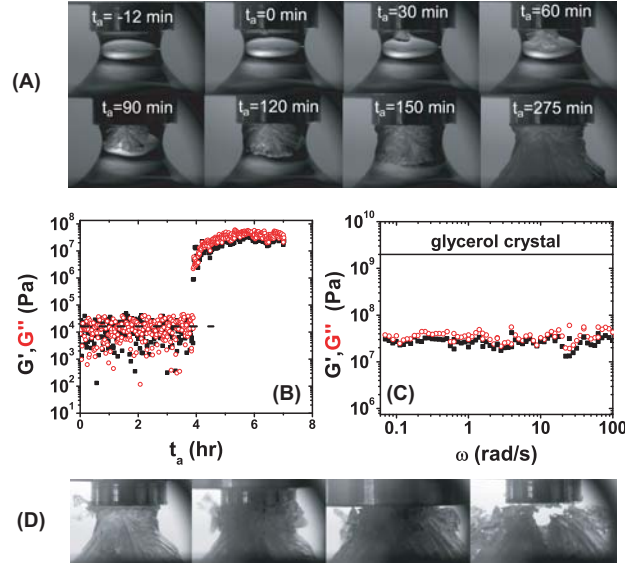
each temperature, we performed an isothermal frequency scan of the complex moduli. As can be seen in Figure 3.5 A, there is an asymmetric peak in  $G''$  at a frequency corresponding to the  $\alpha$ -relaxation time similar to the measurements by Jeong [78] and Schröter et al. [57]. Moreover, the storage modulus  $G'$  at high frequencies levels off at  $3.7 \pm 0.1$  GPa, which agrees well with previous results [57, 75]. Furthermore, we probed the temperature dependence of the relaxation peak in  $G''$ . As shown by earlier experiments [78], the complex moduli can be rescaled on a master curve (see Figure 3.5 B), where the peak frequencies are well fit by a VFTH equation.

Surprisingly, the same temperature protocol, which worked reliably in the Couette geometry to create the solid-like state, could not reproduce the solid-like state of glycerol very well in the plate-plate geometry. Out of 6 trials only one run yielded a solid-like state. In addition, a number of cuvette cells (dimensions  $1 \times 9.5 \times 45$  mm<sup>3</sup>,  $2 \times 9.5 \times 45$  mm<sup>3</sup>,  $5 \times 9.5 \times 45$  mm<sup>3</sup>, and  $10 \times 9.5 \times 45$  mm<sup>3</sup>) filled with glycerol were subjected to the same temperature protocol, and none of them showed a cloudy appearance.

As mentioned above, we did observe once the spontaneous solidification of the



**Figure 3.5:** Relaxation peak of supercooled glycerol near the glass transition ( $G'$  (■) and  $G''$  (○)). (A) Oscillation measurements in plate-plate geometry at strain amplitude  $\gamma = 1.3 \cdot 10^{-4}$  were performed at temperatures  $T=186, 187, 188, 189, 189.5, 190$  and  $191.5$  K. The two arrows indicate the shift of the curves with increasing temperature. (B) Same as (A) with the frequency axis rescaled by the relaxation peak frequency using a VFTH fit:  $\omega_{peak} = 5.7 \cdot 10^{16} \cdot e^{-2856/(T-117)}$  rad/s.



**Figure 3.6:** Emergence of the slushy phase after aging at  $T_a = 240$  K. The same temperature protocol as in Figure 3.2 A was followed. (A) Snapshots of the slushy phase at different times, showing a crystallization-like front moving at  $v = 1.1 \pm 0.2 \cdot 10^{-7}$  m/s. (B)  $G'$  (■) and  $G''$  (○) as a function of time (frequency  $f = 0.1$  Hz, strain amplitude  $\gamma = 7 \cdot 10^{-4}$ ). The noise floor is denoted by the dotted line. (C) Frequency sweep of  $G'$  (■) and  $G''$  (○) at  $t_a = 7$  h, showing a flat frequency response over 3 decades. The line indicates the storage modulus of crystalline glycerol. (D) After the oscillation measurement, a normal force of 10 N is applied and the slushy phase immediately deforms plastically indicating a low rigidity. However, the sample could withstand a normal force of 2 N without plastic deformation.

sample in the plate-plate geometry with our temperature protocol. During that run, a slushy phase formed at  $T_a = 240$  K, as shown in Figure 3.6.  $G'$  and  $G''$  showed a sudden increase once the solidification front spanned the whole gap after about 4 hours aging at 240 K. However, both moduli leveled off around  $3 \cdot 10^7$  Pa — two orders of magnitude below the shear modulus of crystalline glycerol, which is  $2 \cdot 10^9$  Pa (see Figure 3.7).

The measured speed of the solidification ( $v = (1.1 \pm 0.2) \times 10^{-7}$  m/s) front is very close to the growth of the glycerol crystal at the same temperature (see Figure 3.7) in agreement with literature values [79]. In addition, a frequency sweep of  $G'$  and  $G''$  of the solidified sample shows an essentially flat frequency response over three decades in  $\omega$ . The reason for the similar magnitude and fluctuations of  $G'$  and  $G''$  might be slippage at the upper plate. However,

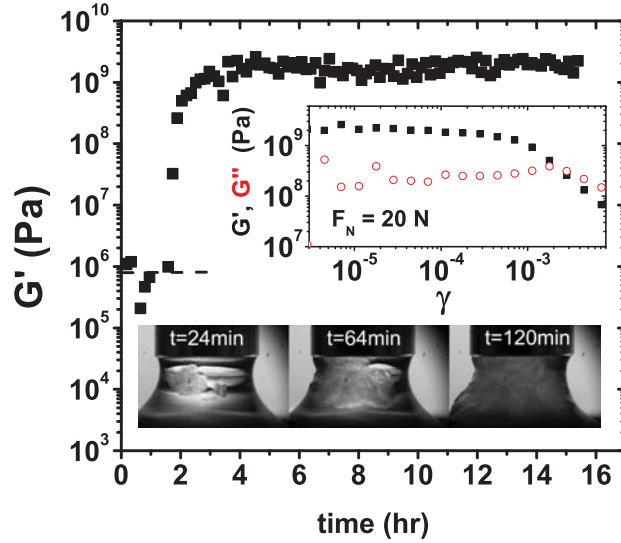
when we applied a moderate normal force of 10 N to the sample in order to reduce slip it immediately deformed and was crushed by the upper plate as shown in Figure 3.6. This is in stark contrast to the crystalline glycerol sample (Figure 3.7) that withstood up to 50 N normal force (the maximum applicable force with our rheometer) without any plastic deformation. This clearly indicates that the slushy phase created through aging has a much lower rigidity than the ordinary crystal.

For comparison, we created an ordinary glycerol crystal and measured its rheological properties. From the previous Couette runs we could harvest crystal seeds that were scratched from the sample before it reached the melting point. These seeds were used to grow large crystals in the freezer at  $T = 253$  K. To compare with the slushy phase, we seeded the glycerol sample in the plate-plate geometry that was quenched at  $T = 240$  K with a few micrograms of the crystal.

By direct imaging we measured the crystallization front that slowly spread from the upper plate through the sample (Figure 3.7) at a speed of  $(9.8 \pm 0.2) \times 10^{-8}$  m/s at 240 K. After two hours, the whole sample has crystallized and the elastic moduli have jumped up. The storage moduli obtained from oscillatory measurements are centered around  $2.2 \pm 0.5$  GPa, which is consistent with previous measurements [59]. A subsequent strain sweep at  $f = 0.1$  Hz at a normal force  $F_N = 20$  N shows that the storage modulus is constant over a range of strain amplitudes, although for larger strain the elastic modulus decreases while the loss modulus increases due to slip. The measurements presented in Figure 3.7 show that we can reliably determine moduli of the solidified glycerol in the relevant GPa range.

### 3.4 Discussion

We have reproduced the solid-like state of glycerol in the Couette geometry at temperatures well above the glass transition as was reported in Chapter 2. We have established that an initial slow cooling period is crucial to create the solid-like state reliably. Perhaps, upon slow cooling, the molecules have enough time to locally rearrange themselves into a configuration that is more favorable for the nucleation of a solid-like structure. This would be more difficult in case of a fast cooling where the molecules are quickly arrested at temperatures close to the glass transition. Once the solid-like structures are formed in the glycerol, the rigidity of the sample increases with time. Both the waiting and the hardening times decrease with increasing aging temperature. However, the maximum moduli we could probe in the Couette geometry are



**Figure 3.7:** Measurement of  $G'$  of supercooled glycerol at 240K in the plate-plate geometry seeded with a crystal.  $f = 0.1$  Hz,  $\gamma = 3.5 \cdot 10^{-6}$ , gap is 2.0 mm. The noise floor is denoted by the dotted line. The inset shows strain sweep ( $G'$  (■),  $G''$  (○)) at 0.1 Hz with applied normal force  $F_N = 20$  N measured 15.4 hours after seeding the crystal. Also shown are snapshots of the growing crystal at different times as indicated. The crystal front grows with speed  $v_c = 9.8 \pm 0.2 \cdot 10^{-8}$  m/s. Once the crystal spans the two plates,  $G'$  rapidly increases.

of the order of  $10^7$  Pa due to the compliance of the measuring tool. The measurements on frozen water indeed confirmed this compliance issue, which also leaves the possibility open that the moduli of solidified glycerol may reach the GPa range.

In order to avoid the compliance issue, we switched to the plate-plate geometry, which also allows us to access the sample optically. Surprisingly, the same temperature protocol, which led to a solid-like state in the Couette cell in every run, did not work reliably in the plate-plate geometry. This is not understood yet. However, it should be pointed out that the Couette cell holds about 7 ml of glycerol and has an effective contact area about  $2000 \text{ mm}^2$ , whereas the plate-plate geometry holds about 0.014 ml of glycerol and the contact surface is about  $9.42 \text{ mm}^2$ . If the formation of the solid-like structure is nucleated from clusters whose probability depends on the volume or/and surface, this nucleation may be very unlikely in the plate-plate geometry.

The nature of the solid-like state remains unclear. It is tempting to conclude

that the solid-like behavior is due to a crystal structure growing in a liquid and giving rise to a viscoelastic linear response in the temperature range of our measurements. This scenario is supported by the observations that the growth rate from a nucleation point and the melting temperature of the solid-like structures are similar to those of the crystal. However, the shear modulus of the slushy phase is two orders of magnitude lower than that of the crystal. Moreover, this slushy material can be easily deformed plastically. If the solid-like parts of the slushy phase are small crystallites, their growth appears to be inhibited. This could be because the crystallites are smaller than the nucleation radius, or because of frustration. These crystallites are loosely organized and coexist with a liquid-like fraction, leading to an overall smaller rigidity than the polycrystals (see Figure 3.7). The images of the crushed sample (Figure 3.6 D) also suggest the existence of such crystallites on a larger scale. However, the crystalline structure of this slushy phase is still speculative at this point and would have to be proved by a structural analysis, e.g., X-ray or neutron scattering. It is reasonable to assume that the solid-like states formed in the Couette cell aged at 220 K, 230 K, and 240 K, after undergoing the same temperature protocol, are also slush-like and will turn into a crystal state upon heating, which is evidenced by the observed melting behavior at the crystal melting point. We believe that the solid-like structure formed by seeding a quenched glycerol sample is a crystal since its shear modulus is of the order of GPa, close to that of crystalline glycerol deduced from sound velocity measurements [59]. Moreover, it can be deformed elastically. To our knowledge, this is the first time the shear modulus of polycrystalline glycerol has been directly measured.

A similar solid-like behavior was found earlier in triphenyl phosphite (TPP), another molecular liquid, well known to exhibit an intriguing glacial state, an apparently amorphous state different from the glass and the ordinary liquid [80–83]. There are some striking similarities between the slushy state of supercooled glycerol and the glacial state of TPP. For example, the rheological experiments on the glacial state of TPP show that the maximum  $G'$  is of the order of  $10^6$  Pa [82], close to  $10^7$  Pa of the slushy phase of glycerol. Both moduli are far below their respective crystalline moduli. Upon heating, the glacial state of TPP turns into a crystal [81, 82], which also happens to the solidified glycerol since it melts at the melting point of crystalline glycerol. The structural description of the glacial state of TPP is still under debate. The evidence that the glacial phase is a mixture of micro-crystallites and non-transformed supercooled liquid came from the measurements performed at higher temperatures (216 – 235 K) [84], whereas the experiments 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, 85]. It would be interesting to do the aging measurements on glycerol at lower temperatures. However, the kinetics associated with this aging process for glycerol appears to be much slower than that of TPP, which makes the experiments e.g. at  $T = 205$  K [74] impractical. It still remains an open question if the scenario of the glacial phase in TPP carries over to supercooled glycerol. If so, the timescales of forming the glacial state are vastly different.

## 3.5 Conclusion

We can reproduce the solid-like state of glycerol in the Couette geometry reliably with our temperature protocol which involves an initial slow cooling period prior to the aging, but not in the plate-plate geometry (except for one run out of six). The observed viscoelastic behavior seems to be related to a crystal-like structure growing in a liquid in the probed temperature range, 220 – 240 K. However, this crystal-like structure can vary considerably in its rheology. The slushy phase, which grew from a nucleation point after slow cooling and subsequent aging at 240 K behaved quite differently from the crystal that was created by seeding a glycerol sample quenched to the same aging temperature. The slushy phase is two orders of magnitude softer and can easily be deformed plastically, whereas the seeded sample has a shear modulus of  $\approx 2$  GPa and can only be elastically deformed. Whether the slushy state of glycerol shares the same structural origin as the glacial state of TPP remains an open question. Nevertheless, the cooling rate emerges as a crucial parameter in studies of glycerol at temperatures near the glass transition and appears to affect the structure and dynamics on the molecular level.

## Acknowledgements

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