SUPPLEMENTARY INFORMATION

Interplay of vitrification and ice formation in a cryoprotectant aqueous solution at low temperature

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I. TEMPERATURE-CONCENTRATION PHASE DIAGRAM OF WATER-GLYCEROL SOLUTIONS

We show in Fig. the phase diagram of water-glycerol solutions where three different ranges of glycerol concentration can be distinguished below the melting line. The range of prime interest for the present study is that in which water is nano-segregated and is part of region II. In region I, water crystallizes upon even rapid cooling (hyper-quenches and alternative techniques may still prevent this to happen, as for pure water) and one can define a line of homogeneous nucleation where micrometer size crystallites of ice are formed when cooling (e.g., by using an emulsification method) the experimental points are displayed as downward pointing (blue) triangles in Fig. and the data can be extrapolated as shown by the dotted blue line. The extrapolation crosses the glass transition line around \( c_g \approx 0.21 \), as is characteristic of bulk water. Unfortunately, we have not covered in detail the range between \( c_g = 0.15 \) and \( c_g = 0.22 \). We find that the phenomenon does appear for \( c_g \approx 0.18 \) but is no longer present for \( c_g = 0.28 \) (see Sec. XI below). An additional criterion is provided by looking at the (positive) difference in macroscopic density between the glass and the high-temperature liquid above melting: this difference is rather constant at high concentration but starts to rapidly decrease around \( c_g \approx 0.22 \). More work is nonetheless needed to more precisely specify the domain where nano-segregation takes place after rapid cooling to a glass and to extend the study to other aqueous solutions.

| sample mass /g | glycerol molar fraction | pseudo-molecule mass /g | \( \sigma_{inc} \) /barn | \( \sigma_{coh} \) /barn | transmission \( \sigma_{coh}/(4\pi) \) /ster.molec |
|-----------------|--------------------------|--------------------------|--------------------------|--------------------------|----------------------------------|
| \( \text{C}_3\text{H}_6\text{O}((\text{O}_3\text{D}_5)\text{D}_5\text{O})\text{D}_5\text{O} \) 1.4011 | 0.178 | 34.31 | 6.2942 | 25.705 | 0.9 | 2.048 |
| \( \text{C}_3\text{H}_6\text{O}((\text{O}_3\text{D}_5)\text{D}_5\text{O})\text{D}_5\text{O} \) 1.4066 | 0.178 | 33.37 | 76.602 | 22.392 | 0.41 | 1.777 |
| \( \text{C}_3\text{H}_6\text{O}((\text{O}_3\text{D}_5)\text{D}_5\text{O})\text{D}_5\text{O} \) 1.4743 | 0.177 | 32.05 | 176.605 | 17.481 | 0.67 | 1.391 |

TABLE S1: Numerical values characterizing the mixtures of glycerol and water for neutron scattering. The sample \( \text{C}_3\text{H}_6\text{O}((\text{O}_3\text{D}_5)\text{D}_5\text{O})\text{D}_5\text{O} \) is only 98% deuterated.

II. DETAILS ON THE CHARACTERISTICS OF THE SAMPLES.

We provide in table S1 some details on the samples: isotopic composition, glycerol molar fraction \( c_g \), mass of an equivalent molecule, where what is called a “molecule” represents a fictitious molecular unit made of \((1 - c_g)\) molecules of water and \( c_g \) molecules of glycerol, incoherent and coherent scattering cross-sections, transmission of the sample on the D7 diffractometer, and finally coherent scattering cross-section per steradian and per molecule.

In Fig. S2 we sketch the molecules of the water/glycerol solutions with their atoms labeled according to Table S1.

III. ABSENCE OF CRYSTALLIZATION UPON RAPID COOLING.

Evidence that the glassy samples with \( c_g \approx 0.18 \) obtained through a rapid enough cooling showed no sign of crystallization is as follows:

The structure factor \( S(Q) \) after a fast quench in liquid nitrogen and prior to annealing shows no detectable signs of crystallization: the peak is at a \( Q \approx 1.75\text{Å}^{-1} \), somewhat higher than that of ice (and of the LDA), and when we observe crystallization upon isothermal anneal-
We show the melting line (open black circles) as well as the glass transition line when no crystallization takes place on cooling (dark blue and light green symbols) for fully deuterated samples. The filled red symbols are our results for fully hydrogenated samples (whose characteristic temperatures are moved up by 3-4 K at melting and 8-10 K at the glass transition\cite{11}). The lines and the extrapolations toward $c_g = 0$ (pure water) are only guides for the eye. As described in the main text, the phenomenology below the melting line is usually divided in 3 regions denoted I, II, and III\cite{12} (see also Fig. S1). In region I water crystallization occurs on cooling (even in liquid nitrogen). The intermediate region II, or even a part of it, is the one of interest here, and we mark by a blue vertical dotted line the concentration which we focus on. We also display as downward pointing blue triangles the temperatures of homogeneous nucleation of ice in the solution\cite{13}, which is expected to shift to the left compared to the actual data, even for such a small typical crystallite size. As also displayed in the figure, we find that the reconstructed structure factor with 1.7nm size crystallites rather corresponds to the experimental data after an annealing of 216min, i.e., when crystallization has indeed started.

Assuming that small ice crystallites of 1nm size crystallites rather corresponds to the experimental data after an annealing of 216min, where water crystallization has indeed started.

One can see that the agreement is very poor and that the putative system with 1.7nm crystallites rather corresponds to the structure after 216min, where water crystallization has indeed started.

Fast enough cooling (10 K/min or faster) therefore seems to completely suppress crystallization for the concentration $c_g \approx 0.18$. 

FIG. S1: Phase diagram of the water-glycerol mixture in the temperature versus glycerol (molar) concentration plane. We show the melting line (open black circles) and filled black circles) as well as the glass transition line when no crystallization takes place on cooling (dark blue and light green symbols) for fully hydrogenated samples. The filled red symbols are our results for fully deuterated samples (whose characteristic temperatures are moved up by 3-4 K at melting and 8-10 K at the glass transition\cite{11}). The lines and the extrapolations toward $c_g = 0$ (pure water) are only guides for the eye. As described in the main text, the phenomenology below the melting line is usually divided in 3 regions denoted I, II, and III\cite{12} (see also Fig. S1). In region I water crystallization occurs on cooling (even in liquid nitrogen). The intermediate region II, or even a part of it, is the one of interest here, and we mark by a blue vertical dotted line the concentration which we focus on. We also display as downward pointing blue triangles the temperatures of homogeneous nucleation of ice in the solution\cite{13}, which is expected to shift to the left compared to the actual data, even for such a small typical crystallite size. As also displayed in the figure, we find that the reconstructed structure factor with 1.7nm size crystallites rather corresponds to the experimental data after an annealing of 216min, i.e., when crystallization has indeed started.

A second argument against the presence of ice in rapidly cooled samples comes from the DSC measurements (see Sec. VII below). When cooled at a fast rate of 10 K/min, still slower than the quench in liquid nitrogen that we use for the study of the structure, no sign of crystallization is detected in the DSC scans on cooling. On the other hand, crystallization is observed by using a slower rate of 2 K/min.

One can see that the fitted peak is too high and shifted to the left compared to the actual data, even for such a small typical crystallite size. As also displayed in the figure, we find that the reconstructed structure factor with 1.7nm size crystallites rather corresponds to the experimental data after an annealing of 216min, i.e., when crystallization has indeed started in the sample.

FIG. S2: Sketch of the molecules with their atoms labeled according to Table S1.
FIG. S4: Sketch of the thermal treatments used in this study. The full blue line and the gray dashed line respectively represent the fast cooling process and the slow cooling one. The symbols indicate some of the points at which structural measurements were taken: The open squares correspond to Fig. S7(left), the triangles to Fig. S7(right), and the circles to Fig. 8 of the main text (liquid I'). The yellow and green horizontal line indicate the annealing stages.

IV. INFLUENCE OF THE THERMAL TREATMENT ON THE STRUCTURAL DATA.

For the neutron scattering study we have considered two cooling protocols, the fast quench in liquid nitrogen already discussed and a slow cooling at 3-6 K/min down to 130 K (6 K/min at the beginning, then 3 K/min below 180 K). The slow cooling is followed by heating to the chosen annealing temperature. The various thermal treatments are sketched in Fig. S4.

The results at 160 K depend on the protocol. We saw that for the fast cooling in liquid nitrogen, crystallization is a slow process taking place over several hours. Alternately, for the slow-cooling protocol, crystallization is much faster. At low temperature, \( T = 130 \text{ K} \), we observe a similar glass structure as for the fast quench, but as soon as one heats the sample, water partly crystallizes: see Fig. S5. Furthermore, whereas we do not detect within our experimental resolution and our analysis hexagonal ice \( I_h \) after the fast quench, it appears to form about 16% of the total ice after the slow cooling. Interestingly, the apparent grain size is similar in the two cases (\( \sim 4-4.5 \text{ nm} \) for slow cooling) but the fraction of water that has crystallized is much smaller after a slow cooling: only 7.5% to be compared to the 21% in the other protocol.

On the other hand, when the samples are heated to 180 K, the structure factor is the same, whatever the protocol: see the right panel of Fig. S7 below.

Fig. S5 illustrates two features of cold crystallization in this aqueous solution. First, the structure evolves with increasing temperature from one dominated by features of the cubic ice with a peak around 1.71 Å\(^{-1}\) to a mixture of cubic and hexagonal ice and finally to a structure dominated by hexagonal ice with its distinctive triplet of main peaks and an additional peak at 2.38 Å\(^{-1}\). The change appears rather gradual, at variance with the suggestion that cubic ice gives way to hexagonal ice as soon as the water grains reach a critical size of about 10-15 nm[11].

Finally, in Fig. S6 we compare the structure factor of \( \text{C}_3\text{D}_5(\text{OD})_3 + \text{D}_2\text{O} \) for \( c_\phi = 0.178 \) obtained by slow cooling of the sample at 3-6 K/min: it is plotted for increasing temperatures from 130 K to 230 K.
V. ROBUSTNESS OF THE STRUCTURE AT 170 AND 180 K VERSUS THE ANNEALING PROTOCOL.

We illustrate here that the states obtained by a fast quench in liquid nitrogen followed by heating and annealing do not depend on the details of the thermal treatment: The structure factors at 170 K are obtained either after a full annealing directly at 170 K or after a full annealing at 160 K followed by heating at 170.3 K; similarly, the structure factors at 180 K are obtained either after a full annealing at 170 K followed by heating at 180.9 K or after a full annealing at 160 K, followed by heating at 180.3 K and finally at 180.6 K. The resulting phases with partially crystallized water are very stable (albeit actually only metastable) and no longer evolve with time: this is shown for $T = 170$ K and $T = 180$ K in Fig. S7.

VI. ANALYSIS OF THE PREFACORS OF THE LOW-Q POROD LAW

We consider the amplitude of the $Q^{-4}$ behavior in the low-$Q$ region of the scattered intensity of the fully deuterated and partially deuterated samples after crystallization at 160 K; see Fig. 1 of the main text. Porod’s prediction states that in the presence of an interface between two phases the scattered intensity at small wavevectors goes as $I(Q) \sim aQ^{-4}$ with

$$a = 2\pi \left( \frac{S}{V} \right) K^2$$  

(1)

where $S/V$ is the area of the interface per unit volume and $K^2$ is the contrast between the two phases. We then fit our data for each sample at low $Q$ to a functional form $aQ^{-4} + b$ with $a$ and $b$ adjustable parameters. The resulting fits are shown in Fig. S5. As can be seen, the fits are good except for the partly deuterated sample $C_3H_2(OD)_3 + D_2O$ for which the Porod behavior is not well pronounced over the probed range of wavevectors due to the presence of additional contributions near and above $0.3\AA^{-1}$.

We would like to rationalize the trend observed between the three different deuterations. However, even when looking at the ratios between the amplitudes $a$, there are still too many unknown parameters. We therefore make some rather crude assumptions to obtain a qualitative or semi-quantitative answer. First, we consider that one of the phase is a mixture of glycerol and water (a proxy for liquid II) with the glycerol concentration $c'_g \approx 0.216$ determined above for the fully deuterated sample (and we take the same value for the other samples) and the other phase is made of ice crystallites. With this assumption, the ratio of the contrasts between two samples denoted 1 and 2 can be obtained as

$$\frac{K_1}{K_2} = \frac{\left[ n_{ps}(c'_g)(c'_g)(\Sigma b)_{g1} + (1 - c'_g)(\Sigma b)_{w1} \right] - n_w(\Sigma b)_{w1}}{\left[ n_{ps}(c'_g)(c'_g)(\Sigma b)_{g2} + (1 - c'_g)(\Sigma b)_{w2} \right] - n_w(\Sigma b)_{w2}}^2$$  

(2)

where $(\Sigma b)_{g\alpha}$ and $(\Sigma b)_{w\alpha}$, with $\alpha = 1, 2$, are the sums of all coherent scattering lengths for glycerol and water in sample $\alpha$; $n_w$ is the number density of cubic ice and $n_{ps}(c'_g)$ is the number density of pseudo-molecules comprising molecules of glycerol and $(1 - c'_g)$ molecules of water. The latter is further approximated by parametriz-
VII. DSC MEASUREMENTS.

We have used two DSC Q-100 from TA instruments, depending on the temperature range explored. One of them was equipped with a compressor and able to go down to 193 K, and the other was equipped with a liquid nitrogen cooling system and able to reach temperatures as low as 140 K.

Two distinct cooling/heating rates have been applied on a fully deuterated sample with glycerol molar fraction of \( c_g = 0.179 \). After melting, the melted water is redissolved in the mixture at 2 K/min, we observe crystallization already on cooling. It is also found on heating, at much lower temperatures than for the faster rate. On cooling, 20% of water crystallizes which leads to a remaining solution with a mole fraction \( c_g \approx 21.3\% \) (and consequently to a glass transition at a molar fraction distinct from the original composition). On heating, an additional 12.7% of water crystallizes, an amount that could be considered as representing “free or interfacial water” after the glass transition. The remaining glycerol/water liquid mixture contains \( \approx 23.1\% \) of glycerol. Note that the spread of melting is much larger, almost over 50 K, for the slower protocol and the maximum of melting occurs at a slightly larger temperature. Both aspects illustrate the larger crystallites and wider distribution of crystallite sizes when the kinetics is slow (higher \( T_m \)). The above results are fully compatible with previous ones obtained in [514].

The procedures are different than those applied during the structural measurements (see above and main text).
and crystallization takes place in different temperature ranges. However, the trends shown by the two sets of measurements fully agree. We always observe water (cold) crystallization upon heating but crystallization on cooling depend on the cooling rate: no water crystallization for fast enough cooling (10 K/min in DSC and down to 3-6 K/min in structural measurements) but crystallization for a slower rate of 2 K/min (DSC).

VIII. ESTIMATE OF THE ICE FRACTION FROM THE HIGH-\(Q\) SCATTERED INTENSITY.

For a molecular liquid, the static structure factor \(S(Q)\) is obtained from the relative positions of the atoms in the sample as the sum of an intramolecular form factor, \(F(Q)\), and an intermolecular contribution, \(D_m(Q)\). \(D_m(Q)\) is the sum of all the interatomic components for atoms belonging to distinct molecules and its Fourier transform gives the intermolecular pair correlation function. The form factor \(F(Q)\) can be calculated from the geometry of the molecules: it is the sum of all the pair correlations between atoms of the same molecule. At large \(Q\), i.e., small distances, only the signature of the molecular form factor prevails in \(S(Q)\): this is illustrated in Fig. S10 for pure water\(^{14,16}\) and pure glycerol\(^{12}\), where one can see that above 2.4\(\AA^{-1}\) the form factor dominates \(S(Q)\) for both liquids. Similarly, for the mixtures of the present study, the value of the measured coherent differential scattering cross section around 2.4\(\AA^{-1}\) only depends on information at the molecular scale. The experimental data around 2.4\(\AA^{-1}\) (see Fig. 2 of the main text) corresponds to the coherent differential scattering cross section in absolute units (barn per steradian and per molecule), which we verify to be equal, before water crystallization takes place, to the calculated value listed in Table 2 within an uncertainty of 0.5%. For each of the two annealing temperatures shown in the upper panels of Fig. 2 (main text), the two red points at 2.4\(\AA^{-1}\) correspond to the signal of the mixture initially prepared at \(c_g = 0.178\) before water crystallization (highest point) and to the mixture after partial crystallization of water (lowest point). As a result of ice formation, the latter signal contains less water molecules and accordingly a higher glycerol molar fraction \(c_g'\). The pseudo-molecule used to compute the coherent differential scattering cross section from the data in Table S1 is thus made of of \([1-c_g\text{ molecule of water} + c_g'\text{ molecule of glycerol}]\) for the initial signal and of \([1-c_g'\text{ molecule of water} + c_g'\text{ molecule of glycerol}]\) for the final one. The difference between the two measured data points at \(Q = 2.4\AA^{-1}\) then gives access to the amount of water that crystallizes and to the average glycerol molar fraction of the remaining uncrystallized mixture.

At 160 K, before any annealing, the initial value \(\sigma_{coh,init}/(4\pi)\) at 2.4\(\AA^{-1}\) is 2.045 barn/(steradian.molecule) with \(c_g = 0.178\). At the end of the crystallization process, the final value \(\sigma_{coh,final}/(4\pi)\) is 1.832 barn/(steradian.molecule). This lower value is due to the decrease of the amount of water molecules in the remaining mixture. The proportion of water in the latter is equal to \(X_{w,final} = (\sigma_{coh,final} - c_g \sigma_{coh,g})/\sigma_{coh,w}\), while the initial proportion is simply \(X_{w,init} = (1 - c_g)\) (where \(w\) and \(g\) refer to water and glycerol, respectively). The fraction of water that crystallizes is therefore \((X_{w,init} - X_{w,final})/X_{w,init}\). At 160 K, 21.3% of water crystallizes, and the remaining mixture has a glycerol concentration \(c_g' = 0.216\). There are several sources of uncertainties in this estimation. One may come from the uncertainty on the effective deuteration rate of the sample (a 1% difference in the deuteration of glycerol from 99% to 98% leads to an error of 0.5% in the estimated fraction of crystallized water). Additional uncertainties can be estimated from the small difference between the \(Q = 2.4\AA^{-1}\) value in full structure factor at the end of the annealing and that in the amorphous component determined through the Rietveld analysis (green curve in Fig. 2 of the main text): this difference however represents less than 1%, which means a 1% uncertainty in the fraction of crystallized water (22.5% instead of 21.3).

With the second thermal treatment corresponding to the slow cooling protocol, crystallization appears rapidly when the sample is heated from the glass to 160 K, and we find that a fraction of 7.5% of water crystallizes. For the partly deuterated sample \(C_2H_6(OH)_2 + \) \(D_2O\) shown in Fig. 1 of the main text, the proportion of water that has crystallized is 19.6% with a glycerol mole fraction of \(c_g' = 0.212\) in the remaining mixture.

At 170 K, a similar analysis provides \(\sigma_{coh,init}/(4\pi)\) \(\approx 2.043\) barn/(steradian.molecule) with \(c_g' = 0.178\) and \(\sigma_{coh,final}/(4\pi)\) \(\approx 1.641\) barn/(steradian.molecule), from which we extract that 39 ± 1% of of water has crystallized with a glycerol mole fraction of \(c_g' = 0.26\) in the remaining mixture. Finally, at 180 K, 53% of water crystallizes and the glycerol mole fraction in the remaining mixture is \(c_g' = 0.32\), not far from the concentration estimated from dielectric measurements by Popov et al\(^{3}\) for liquid II, i.e., 0.38-0.40.

The conclusions drawn here disagree with the results of Murata and Tanaka\(^{3}\) who found, when temperature is rescaled by \(T_g\), a smaller fraction of crystallized water for the fully hydrogenated sample from WAXS experiments. They estimate that the fraction of water that has crystallized is about 12% at 162 K \((T/T_g \approx 1.05)\), 24% at 167 K \((T/T_g \approx 1.08)\) and 34-44% at 170 K \((T/T_g \approx 1.10)\). This is to be compared with what we find here: 21% for \(T/T_g \approx 0.97\), 39% for \(T/T_g \approx 1.03\), and 53% for \(T/T_g \approx 1.09\). These lower estimates are possibly due to a less precise estimation procedure, an insufficient annealing time at the lowest temperatures, or an effectively slower cooling rate.
IX. NEUTRON SPIN ECHO (NSE) RESULTS FOR THE DYNAMICS.

The typical neutron spin echo experiment consists in measuring the polarization at (so-called Fourier) times from \( t = 0 \) ps to 2000 ps. These values are then normalized by the signal at \( t = 0 \) and by the resolution function. Typical curves are shown in Fig. S11 (left) for \( c_g = 0.178 \) and for a range of temperatures in the stable and weakly supercooled liquid (200 to 280 K), which we refer to as liquid I, at the wavevector \( Q = 1.9 \) Å\(^{-1}\) corresponding to the maximum of the structure factor in this range of temperature. A time-temperature superposition curve (right panel of Fig. S11) can then be built and fitted with a stretched exponential function (also known as Kohlrausch-Williams-Watts function) to obtain a more robust value of the stretching parameter \( \beta_{KWW} \): \( f(t) = A \exp[-(t/\tau)^{\beta_{KWW}}] \). The characteristic time \( \tau \) is determined for each temperature to provide the best collapse on the master-curve. The stretching exponent is found to be \( \beta_{KWW} = 0.53 \), a value comparable to that of other molecular glass-forming liquids in their supercooled regime. The experiments were performed on cooling and are reproducible in the temperature range studied.

We have similarly obtained the NSE data for the solutions with \( c_g = 0.28 \) and \( c_g = 0.40 \).

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FIG. S10: Static structure factor of bulk water at 285 K (full dark blue line) and at 127 K in the LDA form (full light blue line) and of bulk glycerol at 300 K (full dark green line) and at 130 K (full light green line and symbols). The shift of the peak of the \( S(Q) \) of water to lower Q’s (blue arrow) is related to the density decrease of water as \( T \) decreases. In contrast, glycerol behaves as a standard molecular liquid with a shift to higher Q’s (green arrow) related to a density increase. The dashed lines, respectively green for water and blue for glycerol, are the calculated form factor \( F(Q) \) in bulk conditions.

FIG. S11: Left: Normalized time-dependent coherent scattering function \( F(Q,t) \) obtained by NSE at the wavevector Q of the maximum peak of the static structure factor, both in the stable and in the weakly supercooled liquid (which we refer to as liquid I): glycerol-water solution with \( c_g = 0.178 \). Right: Rescaling of the data by using time-temperature superposition; the full line is the best fit to a stretched exponential (KWW function).

X. HIGH RESOLUTION \(^{1}\)H NMR.

High resolution \(^{1}\)H NMR allows one to give a clear complementary picture of the specific behavior of water and glycerol on a temperature range that covers the stable and the weakly supercooled liquid regime (liquid I). Fig. S12 presents the spectra of the glycerol/H\(_2\)O mixture (\( c_g = 0.191 \)) as a function of temperature. At higher temperature, two set of peaks are observed, corresponding to the glycerol backbone (around 3.8 ppm) and to the mobile protons (around 5 ppm). This unique peak for the OH groups of glycerol and H\(_2\)O molecules is the signature of a rapid exchange regime between all these exchangeable hydrogen atoms. However, when the sample temperature is decrease below 313 K, this signal splits into two components, corresponding respectively to the OH groups of glycerol (above 6 ppm) and water (around 5.5 ppm). This results from a slowdown of the exchange regime between the groups belonging to glycerol and water. At even lower temperature (280 K), the two types of OH group of glycerol are also clearly split, which means an even longer residence time of all types of OH’s on the glycerol molecules. Going to even lower temperatures, the signal broadens, indicating a strong decrease of molecular mobility. This phenomenon is reversible when heating the sample, with however a temperature hysteresis of around 20 K. In this domain, peak integration shows that half of the signal of water is lost compared to higher temperature, while the signal of glycerol is not affected. This is due to the selective crystallization of a fraction of the water in this mixture below 240 K. Further information can be gained from the measurement of the self-diffusion coefficients by pulsed-field gradient NMR. The specific behavior of each hydrogen group can be selectively measured. The variation of the self-diffusion co-
FIG. S12: Evolution of $^1$H NMR spectra for the the glycerol/H$_2$O mixture ($c_g = 0.191$) as a function of temperature during the cooling stage.

| Temperature (K) | 333  | 325  | 313  | 302  | 298  | 287  | 278  | 266  | 255  | 243  | 231  | 220  | 208  |
|----------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| δ (ppm)       | 0.8  | 1.0  | 1.2  | 1.4  | 1.6  | 1.8  | 2.0  | 2.2  | 2.4  | 2.6  | 2.8  | 3.0  | 3.2  |

FIG. S13: (a) Evolution of the self-diffusion coefficients of the different hydrogen populations (aliphatic for the glycerol backbone, H$_2$O and the two populations of glycerol OH) for the glycerol/H$_2$O mixture ($c_g = 0.191$) as a function of temperature (measurements performed with a diffusion time of 100 ms). The ratio of the diffusion coefficient of water over that of glycerol increases from 2.7 at 295 K to 6 at 250 K. (b) Evolution of the apparent diffusion coefficients with the diffusion time, as measured at 250 K and 300 K.

![Graph showing diffusion coefficients](image)

The evolution of the apparent diffusion coefficients with temperature is presented in Fig. S13. One can see that the ratio of the diffusion coefficient of water over that of glycerol increases from 2.7 at 295 K to 6 at 250 K. At high temperature, the self-diffusion coefficients of all the OH groups have similar values, significantly higher than that of the glycerol backbone. Note finally that the ratio between water and glycerol diffusion coefficients at 295 K is comparable to the value of 2.5 found for a fully deuterated sample at 298 K.

Around 265 K, one can notice that a fraction of the OH groups has the same diffusion coefficient as the glycerol backbone, indicating their longer residence time on glycerol. This slowing-down of the exchange rate is also seen in Fig. S13, where the apparent diffusion coefficients are measured as a function of the diffusion time at two different temperatures. At high temperature, no variations are measured for diffusion time between 20 ms and 500 ms, indicating a Fickian-like behavior. However, at 250 K, the apparent diffusion coefficient of hydroxyl groups is strongly dependent on the diffusion time. At shorter time it is similar to that of the glycerol backbone, while at longer time it is water-like. This characterizes an exchange regime in the range of hundred of ms. The timescale over which nano-segregation locally persists therefore strongly increases with decreasing temperature.

**XI. STRUCTURE OF LIQUIDS I AND I’.**

First we show that water in the nano-segregated liquid I’ bears no resemblance to its high-density amorphous form and rather look like the low-density form: This is unambiguously seen from Fig. S14.

We also provide additional neutron-scattering structural data illustrating the difference between what we refer to as liquid I and liquid I’ for the glycerol-water solution with $c_g \approx 0.18$.

In Fig. S15, we display the structure factor $S(Q)$ of liquid I at 260 K above melting and of that of liquid/glass I’ at 130-160 K (prior to water crystallization). The maximum shifts from $1.83 \, \text{Å}^{-1}$ at 260 K to $1.75 \, \text{Å}^{-1}$ at 130 K, a trend which is typical of bulk water (see Fig. S10), and one can see an intensity increase at lower $Q$’s at 170 K. These features are indications of an increased nano-segregation of water during the fast cooling (and during the subsequent heating to the annealing temperature, prior to ice formation). Further support comes for the fact that while the main peak follows the trend of bulk water the average liquid density...
FIG. S15: Structure factor $S(Q)$ of liquid I at 260 K above melting (green symbols) and of glass/liquid I' at 130 K and 160 K (blue symbols) for the $c_g = 0.178$ glycerol-water solution. This illustrates the increased nano-segregation effect during cooling.

FIG. S16: Comparison of the structure factors of liquid I (green line) and liquid I' (blue line) for $c_g = 0.178$ (same data as in Fig. S15) with those of liquid I (orange squares and associated line) at 275 K and of glass/liquid I' at 130 K (brown squares and associated line) for a higher glycerol concentration $c_g = 0.28$. although a detailed comparison is difficult because of the change in the weighting of the partial structure factors between $c_g = 0.18$ and $c_g = 0.28$, one can notice that the position of the main peak of liquid I' is around 1.75 Å$^{-1}$ and does not seem to change much with concentration. The variation with temperature (i.e., between liquid I and liquid I') of the peak position is significant and goes to lower $Q$ as $T$ decreases for $c_g = 0.18$ whereas it is negligible for $c_g = 0.28$. The displacement to lower $Q$ tracks the nano-segregation of water, and this effect disappears as $c_g$ increases. We conjecture that at even higher glycerol concentration, for $c_g \gtrsim 0.38$, the peaks shifts to higher $Q$ as $T$ decreases, as seen in standard molecular liquids (see glycerol in Fig. S10).

The larger temperature difference for the glass transition, conforms to the behavior of a conventional molecular liquid and increases between the high-temperature liquid I and the low-temperature liquid I' 

Finally, we also show a comparison of the evolution of the structure of between liquid I (above melting) and glass/liquid I' at $c_g \approx 0.18$ and at a higher glycerol concentration $c_g = 0.28$. Although a detailed comparison is difficult because of the change in the weighting of the partial structure factors between $c_g \approx 0.18$ and $c_g = 0.28$, one can notice that the position of the main peak of liquid I' is around 1.75 Å$^{-1}$ and does not seem to change much with concentration. The variation with temperature (i.e., between liquid I and liquid I') of the peak position is significant and goes to lower $Q$ as $T$ decreases for $c_g \approx 0.18$ whereas it is negligible for $c_g = 0.28$. The displacement to lower $Q$ tracks the nano-segregation of water, and this effect disappears as $c_g$ increases. We conjecture that at even higher glycerol concentration, for $c_g \gtrsim 0.38$, the peaks shifts to higher $Q$ as $T$ decreases, as seen in standard molecular liquids (see glycerol in Fig. S10).
which is 10-12 K for pure water and slowly decreases as $c_g$ increases, stems from quantum effects observed at low temperatures: see Gainaru, C., Agapov, A. L., Fuentes-Landete, V., Amann-Winkel, K., Nelson, H., Köster, K. W., Kolesnikov, A. I., Novikov, V. N., Richert, R., Böhmer, R., Loerting, T., and Sokolov, A. P. Isotope effect in the glass transition of water, *PNAS* **111**, 17402-17407 (2014).

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