Supporting information for: Changing Hydrogen-bond Structure during an Aqueous Liquid-liquid Transition investigated with Time-resolved and Two-dimensional Vibrational Spectroscopy

Jeroen R. Bruijn, Tibert van der Loop, and Sander Woutersen*

Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

E-mail: s.woutersen@uva.nl

Experimental details

The samples were prepared as follows. Glycerol (99.9%, Sigma-Aldrich) is mixed with water with a molar fraction of glycerol of $c = 0.172$. The 0.828 molar fraction of water consists of 95% water (99.9%, Sigma-Aldrich) and 5% D_2O (99.96%, Eurisotop). Note that water and glycerol have very fast ($\ll$s) OH/OD exchange, as can be observed directly from the OH/OD bend modes (see below). After shaking for 1 minute the sample is sonicated for 3 minutes, after which a homogeneous mixture is obtained. Next, 1 μl of sample is placed between two CaF_2 windows separated by a $\sim$10 μm spacer. We ensure that the sample does not make contact with the spacer, since the spacer functions as a crystallization site when supercooling the sample. The sample is placed in a cryostat (Optistat DN1704, Oxford Instruments), in which the temperature can be actively controlled by cooling

with N_2 and heating by an electronically controlled heat exchanger. Once inside the cryostat, the water/glycerol mixture is cooled down rapidly to either 171 or 195 K to obtain a 2DIR spectrum of Liquid I. Conventional FTIR spectra are taken every 1 or 2 minutes during the transition to track the changes in absorption during the LLT. The FTIR beam diameter in these time-resolved experiments is several mm, large enough to average over any spatial inhomogeneities in the sample. Once the transition is finished, the 2DIR spectrum of Liquid II is measured. Ice-Glycerol formed at 195 K is cooled down to 171 K after the transition is complete for better comparison. A Perkin-Elmer Spectrum-Two FTIR spectrometer is used to measure the FTIR spectra. A stationary camera (NEX5, Sony) is used to observe the phase transition on a macroscopic level (Figure 1A of the main text). The 2DIR measurements were performed using a setup described in detail previously.[S1] The probe pulses have a spectral width of $\sim$150 cm^{-1}, the pump pulses of $\sim$7 cm^{-1}; the pump-probe cross correlation is approximately single-sided exponential with a FWHM of 800 fs at the position of the liquid layer. The polarizations of pump and probe were perpendicular. As a consequence the pump-scatter contribution to the pump-probe signal was very small (about 1% or less of the pump-probe signal); it was eliminated by subtracting the signal measured at large negative pump-probe delay. The acquisition time to obtain the 2DIR spectra was slightly less than 9 minutes for liquid I, 42 minutes for liquid II, and 34 minutes for ice+glycerol.

OH/OD exchange between water and glycerol

In refs. [20] and [21] of the main text it was assumed that no OH/OD exchange takes place between water and glycerol. Since such an absence of OH/OD exchange would significantly influence the interpretation of our results, we performed test experiments to investigate this issue. To check if OH/OD exchange between glycerol and D_2O occurs we first mixed glycerol-H with excess D_2O. Figure S1 shows the NMR spectrum of glycerol-H in $CDCl_3$, where the contributions of the OH groups on glycerol can be identified at $\sim$2.5 and $\sim$1.8 ppm. Upon addition of D_2O and shaking the sample for a few seconds, the integral of the CH protons remains the same (located at $\sim$3.7 ppm),

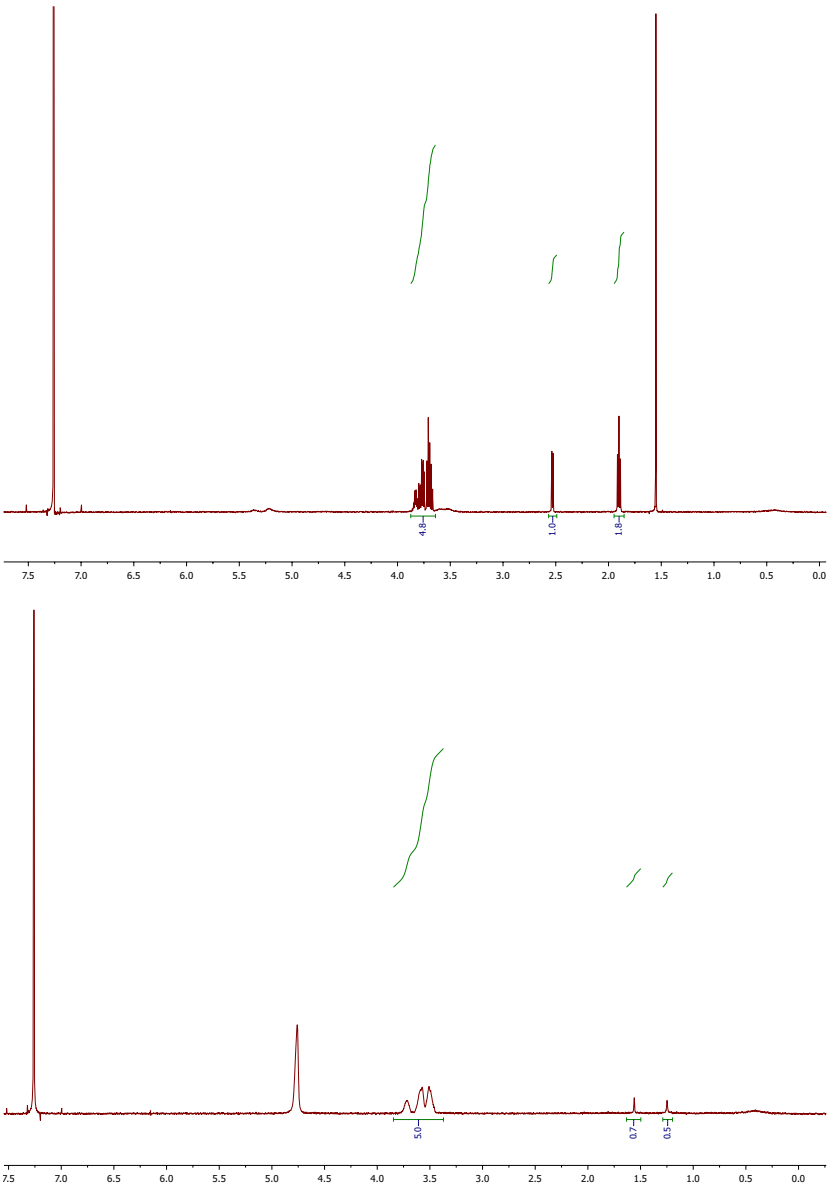

Figure S1: NMR Spectrum of glycerol in $CDCl_3$ (top); the same, after addition of D_2O (bottom).

while the signals of the OH protons have disappeared. The addition of excess D_2O causes the H contribution to be mainly from HDO located at 4.7 ppm.

In addition, FTIR absorption spectra were taken of anhydrous glycerol, neat D_2O, and a mixture of 50/50 volume percent D_2O and glycerol, as displayed in figure S2. The FTIR spectrum of D_2O shows one main absorption at 1210 cm^{-1} due to the DOD-bending mode. There is a broad contribution from the combination mode of the bend and libration mode of D_2O at $\sim$1600 cm^{-1}.

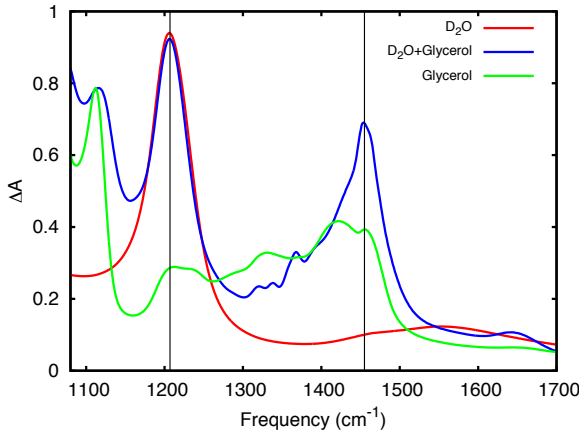

Figure S2: FTIR spectra of anhydrous glycerol (green curve), anhydrous D_2O (red curve) and a mixture of 50/50 volume percent D_2O and glycerol (blue curve). The absorption at 1210 cm^{-1} is associated with the D_2O bending mode, the absorption at 1450 cm^{-1} with the HDO bend mode. The appearance of the latter upon mixing glycerol and heavy water shows that the hydrogen atoms on the glycerol molecule exchange with the deuterium atoms of D_2O.

The small shoulder at $\sim$1450 cm^{-1} is due to the HDO bending mode of a very small amount of HDO present in the D_2O sample. The absorption spectrum of glycerol-H shows a combination of absorption peaks in the frequency range from 1150 to 1500 cm^{-1} that are associated with CH_2 and COH bending modes.

After mixing 50/50 volume percent of D_2O and glycerol-H a homogeneous mixture is obtained within seconds. The IR spectrum of this mixture shows the same absorptions as observed for glycerol-H and D_2O, but the large increase of the peak at 1460 cm^{-1}, the HDO bending mode, shows that the OH hydrogen atoms on glycerol have exchanged with the deuterium atoms on D_2O. The newly risen peak at 1650 cm^{-1} is associated with the HOH bending mode, indicating that some of the D_2O molecules have exchanged both their deuterium atoms with glycerol. The NMR and FTIR data demonstrate that the exchange between D_2O and glycerol occurs on a time scale of seconds. Consequently, the OH/OD ratio for water is the same as for glycerol in our experiments, and the OD-stretch (and OH-stretch) spectra contain contributions of both water and glycerol.

References

(S1) Viga, A. H.; Shaw, D.; Woutersen, S. *J. Phys. Chem. B* **2010**, *114*, 15212–15220.