Supporting Information

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SI Methods

Small-Angle X-Ray Scattering. The SAXS experiments were performed at beamline 4–2 at the Stanford Synchrotron Radiation Lightsource (SSRL) using a beam energy of 11 keV and an optical fiber coupled CCD detector (MarCCD165) (1). For water measurements, a quartz capillary sample holder with a diameter of 1.5 mm was integrated into the vacuum setup eliminating the need for additional windows between the beam defining slit and the exit window of the flight path. The sample holder, detector and the path in between were placed in a vacuum of 1×10^{-3} torr to reduce the background scattering. For CCl_4 measurements, 400-μm thick CCl_4 is sandwiched by two 10-μm thick mica windows. Both water and CCl_4 sample devices were connected to a constant temperature bath, within which water was circulated to maintain and control the sample temperature. Data were collected at three different sample-detector lengths, covering the range of the scattering vector, defined as $Q = 4\pi\sin\theta/\lambda$, between 0.04 to 0.78 Å^{-1}, where λ is the wavelength and θ is one-half of the scattering angle. To reduce the possibility of radiation damage, the data were continuously collected for 10 frames with 1 min each and all scattering images were averaged afterward. The scattering curves have been corrected for the primary beam intensity, absorption and detector readout noise. The scattering of the empty capillary at each temperature was measured separately and subtracted. The water scattering was observed to be $\approx 80\%$ of the total scattering at $Q = 0.05$ Å^{-1}. Thus, the capillary scattering subtraction is very reliable.

X-Ray Raman Scattering. The XRS experiments were performed at beamline 6–2 at SSRL, equipped with a Si (311) double crystal monochromator. Raman scattering was analyzed with a high-energy-resolution multicrystal analyzer in a Rowland geometry (R = 1 m) using 14 Si (440) analyzer crystals (100 mm in diameter) at a fixed Bragg angle of 88°. This setup selects 6.46 keV photons with a resolution of ≈ 0.3 eV and momentum transfers of $Q = 2.6 \pm 1$ Å^{-1}. The monochromator energy was scanned from 6,990 to 7,060 eV with a flux of $\approx 3 \times 10^{12}$ photons/sec focused down to a 0.1×1 mm^2 spot size. The overall energy resolution amounted to 0.5 eV full width at half maximum (FWHM). Approximately 40 scans were averaged for each temperature with a total count of $\approx 15,000$ to 20,000 per averaged spectrum. Energy calibration was checked periodically by recording the elastic peak before and after a given temperature point. To correct for energy drift between the elastic peak calibration points, for each temperature, the scans were energy-calibrated to the noncalibrated sum of each series, resummed and calibrated again. We estimate that the relative energy calibration is accurate to within $\pm$ 0.025 eV. The water was flowed through an aluminum cell with a 5×5 mm^2 Si$_3$N$_4$ window of 1-μm thickness. The X-ray path from the beampipe to the detector (including the whole sample cell) was kept in 1 atm He to avoid background scattering from air.

X-Ray Emission Spectroscopy. The O 1s XES measurements (2) were performed with a total energy resolution of 0.35 eV at BL17SU at SPring-8, Japan. A 150-nm-thick Au-coated Si$_3$N$_4$ window was used to separate the liquid flow from the high vacuum and to transmit the incoming and outgoing soft X-rays; coating the window with Gold and using a sample flow rate of 20 mm/sec were important to eliminate radiation damage. For precise alignment of the XES spectra of the three forms of water, i.e., ice, liquid and gas phase water, we used the same XES spectrometer while replacing the liquid cell by other sample mounts for ice and gas phase water.

Molecular Dynamics Simulations. The SPC/E (3) simulations were performed using the parallelized MD code MDynaMix5.0 (4). We used simulation boxes with 40,000 molecules in the NVT ensemble, resulting in box lengths of ≈ 106 Å and using the experimental densities at each temperature. An NPT simulation at 25 °C was also performed and seen to give the same small-angle scattering as in the NVT ensemble. Simulation boxes with 5,000 molecules were first equilibrated and then replicated periodically into $2 \times 2 \times 2$ supercells. These large boxes were further equilibrated for at least 50 ps to eliminate artificial periodicity. Because the oxygen atom positions in the simulations correspond very well to the center of mass of the molecular charge densities, the oxygen–oxygen pair-correlation function, $g(r)$, should contain all of the information on long-range heterogeneities if present. The $g(r)$ was calculated up to $r_{max} = \sqrt{2} \cdot L$ Å (where L is half the box length) by using interatomic distances out to the edges of the simulation box, and then Fourier transformed to obtain the partial structure factor according to

$$S(Q) = 1 + 4\pi\rho \int_0^{r_{max}} \varpi(r) r^2 [g(r) - 1] \frac{\sin Qr}{Qr} dr, \qquad [4]$$

where ρ is the average number density of water at the given temperature and $\varpi(r)$ is a modification function that replaces truncation ripples in the FT with a single unphysical bell-shaped bump at small Q the width of which is inversely proportional to the maximum distance used in $g(r)$ (5). Finally, the derived $S(Q)$ were averaged over between 300 ps (67 °C) and 440 ps (5 °C) in the production stage of the simulations.

As an independent check of the validity of our simulated small-angle scattering intensity, we compared the isothermal compressibility obtained according to Eq. **1** after extrapolating the simulated S(Q) down to $Q = 0$ with results obtained by the fluctuation formula

$$\chi_T = \frac{\langle V^2 \rangle - \langle V \rangle^2}{k_B T \langle V \rangle}. \qquad [5]$$

Very long simulations in the NPT ensemble are required for convergence in χ_T. Here, simulations with 512 SPC/E water molecules were performed at 5, 25, 47 and 67 °C for 12 ns, and the last 5 ns were used to calculate χ_T.

1. Smolsky IL, et al. (2007) Biological small angle X-ray scattering facility at Stanford Synchrotron Radiation Laboratory. *J Appl Cryst* 40:453–458.
2. Tokushima T, et al. (2008) High resolution X-ray emission spectroscopy of liquid water: The observation of two structural motifs. *Chem Phys Lett* 460:387–400.
3. Berendsen HJC, Grigera JR, Straatsma TP (1987) The missing term in effective pair potentials. *J Phys Chem* 91:6269–6271.
4. Lyubartsev AP, Laaksonen A (2000) M. DynaMix—a scalable portable parallel MD simulation package for arbitrary molecular mixtures. *Comput Phys Comm* 128:569–589.
5. Waser J, Schomaker V (1953) The Fourier inversion of diffraction data. *Rev Mod Phys* 25:671–690.