Water structure: Methods

Several independent methods have been used to investigate the structure of liquid water and aqueous solutions. Although each method is often promoted as producing accurate results, the different methods produce conflicting conclusions. This not only generates academic disputes but also, and more importantly, causes general confusion and bewilderment leading to scientific disorientation.

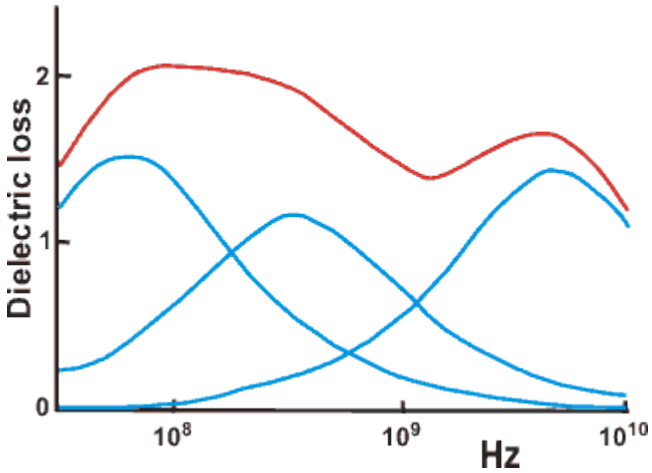
Why different methods give different water structures?

Each method produces data but this data does not directly give the structure of water. It has to be interpreted. Any analysis depends on a theoretical base to interpret the data. More importantly, the results are only as good as this theoretical base, however excellent the quality of the data collected. In addition, different methods give information at particular time-, or size-scales and so may naturally differ.[a] Here I show the principles behind the most important analytical methods (Dielectric spectroscopy, Diffraction methods, Models, Nuclear magnetic resonance, Physical properties,Vibrational spectra, X-Ray spectroscopy) and the main assumptions made to interpret their resulting data. The size and time scales for the different techniques are given below:

Timescales for different methods

Structure	Method(s)	Time and size scales
Instantaneous	Pump-probe laser, X-ray absorption	~10^{-15}s, single molecule
Vibrationally averaged	Infrared, Raman, models	~10^{-12}s, single molecule - local cluster
Diffusionally averaged	Neutron scattering, NMR shift	~10^{-9}-10^{-6}s, local cluster
Probability	X-ray diffraction, thermodynamics	> s, local cluster

Instantaneous structures show the actual positions and orientations of molecules, but these will be disordered relative to each other due to vibrational and linear and rotational diffusional motions; thus they will not show, or will show only poorly, any long term or extensive order in the system. Diffraction data shows long term order but cannot determine whether the order is due to the same molecules being present in the same positions throughout or whether there are simply 'favored' positions with the molecules exchanging rapidly between them. [Back to Top]



Dielectric spectroscopy

Dielectric spectroscopy [950] measures water tumbling and can detect water quantitatively in different clusters if they are held by different average hydrogen bond strengths. The dielectric loss is determined over a wide frequency range (kHz-GHz). Slow tumbling (~ ns) is due to tightly bound water whereas fast tumbling (~ ps) is due to loosely bound water. Opposite is shown an example spectrum of a protein solution [785], where the non-bulk water dielectric loss spectrum (red) can be divided into three underlying Gaussian peaks.[c]Difficulty remains in unambiguously assigning the molecular structures that produce these peaks. Dielectric spectroscopy does seem to show that long range interactions (~0.1 mm) are present in water [1630]. [Back to Top]

Diffraction methods

Diffraction methods measure the time-averaged distances between the atoms of water molecules but cannot directly determine the cluster geometry. X-ray diffraction is sensitive to the concentration of electrons. Even though there are twice as many hydrogen atoms as oxygen atoms in water, these electron-poor atoms contribute little with 85% of the data being provided by the $g_{OO}(r)$ radial distribution function and the remainder from the $g_{OH}(r)$ radial distribution function [1024]. Neutron diffraction is sensitive to nuclei but needs mixtures of D_2O, HDO and H_2O and so produces less precision and accuracy as such mixtures structure water differently from pure H_2O. It gives $g_{OO}(r)$, $g_{OD}(r)$ and $g_{DD}(r)$ radial distribution data, which does allow some, if incomplete, orientation information to be extracted for neighboring water molecules. Electron diffraction can only be used on thin films due to its strong scattering. Recent wide-angle X-ray diffraction has confirmed the existence of two distinctly different hydrogen-bonded environments in liquid water [1755].

The use of diffraction methods for studying the hydration structure of alkali ions has been reviewed [1062]. Diffraction data may be refined by fitting water model simulations [1245]. The resulting fit, however, is insensitive to the model chosen [888] such that optimizing the model to the fit does not, perforce, produce a better model. Diffraction results are discussed further elsewhere. Diffraction methods give data little different from bulk water beyond the first hydration shell where other methods indicate changed structure [1427].

Diffraction methods are excellent for testing models of water structure but less good for delivering the real structure of water due to the difficulty in obtaining orientation information and correlations at intermediate distances. [Back to Top]

Modeling

Many molecular models for water are described on another page of this site. An underlying assumption for these models is that the structure of liquid water may be modeled using just two-body effects (that is, molecule A affects molecule B independently of molecule C affecting molecule B) and do not include any contribution from any covalency in their interaction. Models for liquid water have, so far, proved to be poor predictors for physical and molecular properties outside those used in their development and parameterization. Modeling studies may result in a picture of an 'average' liquid water molecule, which may be misleading as such 'average' structures do not exist in most ice phases and may not be relevant to liquid water. [Back to Top]

Nuclear Magnetic Resonance (NMR)

Water contains three isotopes of use in NMR.[d]

Properties of hydrogen nuclei

NMR, H_2O atoms	^1H	^2H	^{17}O
Spin states	+1/2, -1/2	1, 0, -1	5/2, 3/2, 1/2, -1/2, -3/2, -5/2
Relative sensitivity at natural abundance	1	1.4×10^{-6}	1.1×10^{-5}
Gyromagnetic ratio, rad T^{-1} s^{-1} *	26.7522×10^7	4.1066×10^7	-3.6281×10^7
Frequency at 2.3488 T (MHz) *	100	15.3506	13.5565

* data from WebElements.

The ^1H and ^2H NMR spectra can be used to quantify the deuterium atom content of H_2O, HDO, D_2O mixtures from the natural abundance to nearly 100% [1817]. As it has been reported that magnetic fields may alter the clustering of water ([192, 485, 647]), then the results of NMR must be considered with this possibility in mind. Another weakness to the use of NMR is that only *ortho*-water is NMR-active, para-water having no intrinsic magnetic moment [835].

NMR analysis generally gives reduced information due to proton exchange and movement of water between environments. The average proton residence time on a water molecule is about a millisecond at 25 °C and pH 7, being less than that at other pHs or higher temperatures. Therefore, only single, population weighted averaged, ^1H, ^2H or ^{17}O NMR peaks are generally seen in aqueous solutions. The exception to this is where separate non- (or very slowly) exchanging pools of water molecules are found.

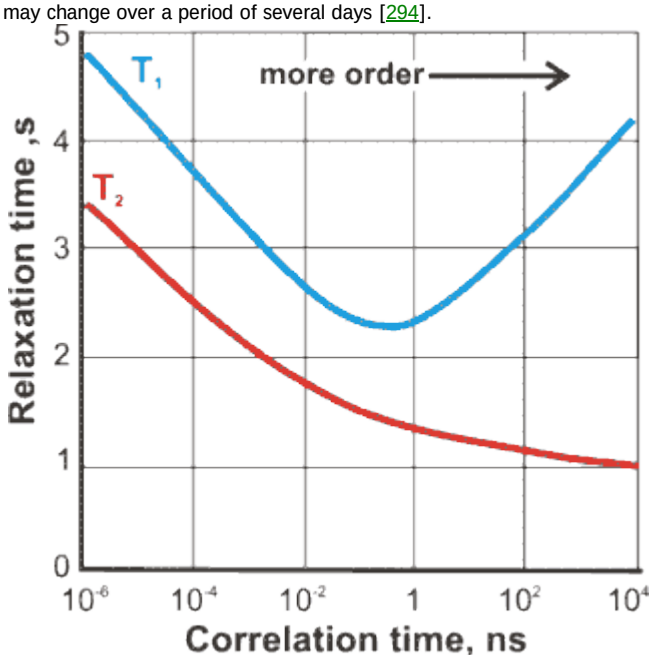
Water's NMR chemical shifts (^{17}O and ^1H) are affected by ions in solution with increasing ionic radius generally causing an increased deshielding effect on the water oxygen atoms but a decreased deshielding effect on the water's protons [781]. The effects are linearly concentration dependent with a change in slope above the concentration that causes a minimum in the specific heat (~ 2-3 M) [556]. Here, the charge density effect (greater charge density causing greater deshielding), as seen in the division of ions into kosmotropes and chaotropes is less important than the ionic size effect [781]. As larger ionic size results in an increased number of first shell water molecules interacting with the ion's field within the clathrate surroundings, the apparent shielding change may be due to an increased effect on the average shift.

Both ^1H and ^{17}O NMR peaks shift to downfield (greater ppm) with increasing hydrogen bond strength; in the case of ^{17}O, the donor O shift increasing greater than the acceptor O reduces. However, only 'averaged' water environments may be obtained. Where several aqueous environments exist, the ^1H NMR spectrum (mainly just one broad peak) may be deconvoluted into Gaussian sub-bands due to differing degrees of hydrogen-bonded water with greater chemical shift (that is, ppm) indicating stronger hydrogen bonding and greater tetrahedrality of the water clustering [851].

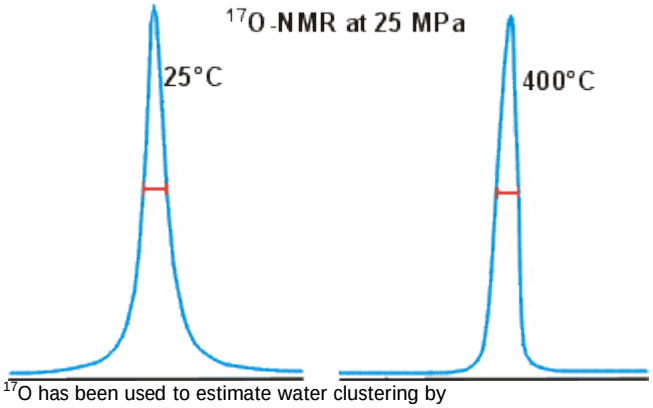
NMR has been used to estimate changes in water's hydrogen bond lengths and strengths [458]. Such investigation makes use *ab initio* calculations to interpret changes in the longitudinal relaxation T_1 (see below) of HDO in D_2O solution by assuming (somewhat incorrectly) that this mixture has a similar structure to pure H_2O.

The NMR signal undergoes two relaxation processes; spin-lattice (longitudinal) relaxation resulting in the decay of the spin distribution (T_1) and spin-spin (transverse) relaxation (T_2) resulting in the loss of the spin coherence. T_2 is inversely proportional to the width (at half height) of the NMR peak. T_1 is 3.6 s at 25 °C due mostly to dipole-dipole couplings [430]. As T_1 reduces with increasing dissolved molecular oxygen concentration (due to its paramagnetic nature) [782] and dissolved oxygen concentrations are expected to be greater in more hydrophobic

environments near hydrophobic surfaces, dissolved oxygen is capable of giving rise to artifacts in the NMR spectra of water. Such affects may change over a period of several days [294].



Shorter T_2 values indicate less molecular mobility; the ^1H T_2 of water at 25 °C, viscous water and ice at 0 °C are about 3 s, ~1 ms and ~5 µs respectively. However, the T_2 relaxation times are also reduced by exchange reactions, with for example, proteins carboxyl, hydroxyl and amine groups. Generally only single, population weighted averaged, ^1H, ^2H or ^{17}O relaxation rates are seen in aqueous solutions. The exception to this is where separate non- (or very slowly) exchanging pools of water molecules exist such as occur in porous media including biological tissue and many foodstuffs. T_1 and T_2 are approximately equal for liquid water, both dropping with increased hydrogen-bonded clustering (for example, increased viscosity) under normal conditions. However, as the degree of tetrahedral clustering increases further, T_1 increases as T_2decreases [581]. T_1 thus shows a minimum at intermediate viscosity, at the NMR resonant frequency, whereas T_2 decreases continuously with increasing viscosity. Note that NMR relaxation is a non-equilibrium kinetic event and cannot give thermodynamic quantities such as water activity.



^{17}O has been used to estimate water clustering by means of NMR line-width measurement where strongly clustered water gives broader peaks.
However this reasoning is faulty and the data is often imprecise and probably affected by dissolved O_2 and pH; see also Paul Shin's critique of this method.

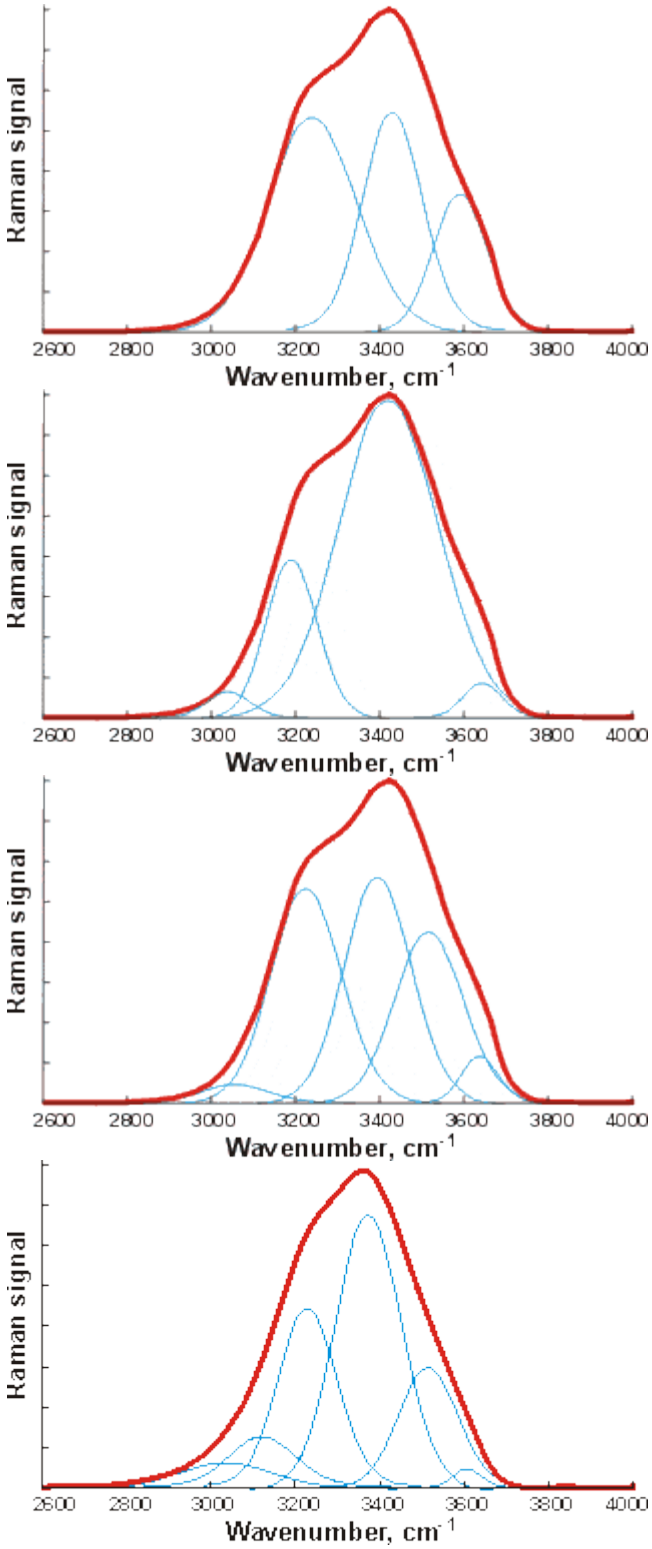
Shown opposite are the ^{17}O NMR peaks for water at widely different temperatures, showing how little the width changes even under so different conditions [783].

Nuclear Overhauser effects (NOEs) may be used to identify relatively static water molecules within the first hydration shell (<~0.5 nm) around biological molecules [784].

Apparent diffusion rates of water are measured using magnetic field gradients and may be incorporated into magnetic resonance images (MRI). As diffusion is restricted in more extensive aqueous clusters, this method can differentiate different pools of water by their mean diffusion rates. [Back to Top]

Physical properties

Physical properties such as viscosity, compressibility, speed of sound, heat capacity, refractive index, thermal conductivityand surface tension can all be used to gather information concerning the structure of water. The interpretation of this data is not clear-cut, however. Note that only the surface properties, not the bulk properties, are discovered using surface tension. The surface structure of water is very different from the bulk structure and may mislead. [Back to Top]



Vibrational spectra

Water gives complex infrared and Raman spectra [1738]. Analysis of these, together with changes caused by variations in temperature and/or pressure, or the presence of solutes, is thought capable of providing detailed information concerning liquid water structuring. Liquid water spectra reportedly corresponds to linear mixtures of just two contributing forms [1738].[b] Due to peak broadening caused by the varying interactions between neighboring molecules, the spectra may be analyzed by supposing a number of Gaussian-shaped[c] vibrational absorptions underlying the complex peaks. Difficulties in the analysis concern determining how many absorption peaks are present and what are the molecular origins for these vibrations. Clearly the more vibrations that are thought to contribute to a complex absorption peak, the better may be any produced fit.

The peak at about 3400 cm^{-1}, mainly due to symmetric and asymmetric stretching, has received considerable attention. It appears to consist of a small number of overlapping peaks but may be far more complex. The assignment of symmetric, asymmetric stretch and bending overtone is usually discarded in favor of the assumption of combined stretch absorptions shifted due to local hydrogen bonding arrangements. However and importantly, there is no consensus as to the number of underlying absorptions with different researchers using three, four or five absorptions, as are shown indicatively opposite.

More confusion in the analysis is due to the lack of a consensus view as to what these underlying absorptions represent. It is unclear whether the number of hydrogen bonds are important [786], or is it the type of hydrogen bond (single, bifurcated or trifurcated) [573], or is it the hydrogen bond bending angles [439], or perhaps the nature of the formed or broken donor and acceptor hydrogen bonds [699b], or the length of the hydrogen bonds [1318]. Each of these has been clearly argued (for example, see below) but the contributing vibrational peaks must contain all of these (and other) contributions. It is now believed that analysis involving individual water molecules and gaussian peaks may be inherently flawed as the absorption bands also reflect coherent vibrational transfer involving several to many water molecules [2003].
Further confusion of the Raman spectroscopy is that the peaks in the 3000-3700 cm^{-1} range are affected by the Raman excitation wavelength [1536]. In particular, the component at ~3200 cm^{-1} is in resonance with visible red light from a vibrational overtone.

Assignments from different authors

Peak[a]	**Water's assigned hydrogen bond molecular linkages**			
0	unlinked	1 bifurcated[b], 1 trifurcated[c]	2 H-bond, donors broken	3636 cm^{-1}, unlinked
1	1 H-bond	2 bifurcated	2 H-bond, acceptors broken	3572 cm^{-1}, 3 H-bond, acceptor broken
2	2 H-bond	1 O-H H-bonded, 1 bifurcated	3 H-bond, donor broken	3430 cm^{-1},2 H-bond, acceptor and donor broken
3	3 H-bond	Both O-H are weakly H-bonded	3 H-bond, acceptor broken	3220 cm^{-1}, 4 H-bond
4	4 H-bond	Both O-H are strongly H-bonded	4 H-bond	3014 cm^{-1}, 3 H-bond, donor broken
Ref.	[786]	[573]	[699b]	[1925]

[a] From high to low wavenumber; 0 = greatest wavenumber.
[b] The O-H hydrogen bond is shared between two accepting water molecules.
[c] The O-H hydrogen bond is shared between three accepting water molecules.

A thorough and careful analysis of the combination band centered at about 5260 cm^{-1} failed to distinguish the substructures that correlate with the vibrational sub-bands [909].

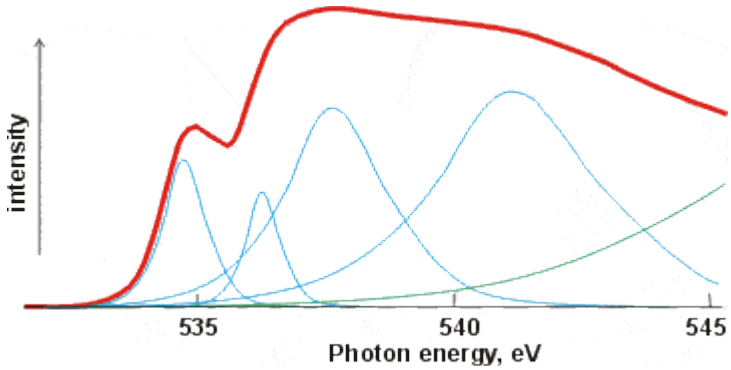
The use of a pump-probe laser using 70-fs pulses at 3350 cm^{-1} (O-H stretch) and detecting the resultant molecular vibrations within the hydrogen-bonded clusters over a range of frequencies with time shows efficient energy redistribution [750]. However, this method is not yet able to show structuring details.

Two-dimensional Raman spectroscopy has been shown to provide information concerning water's intermolecular dynamics and may eventually provide data on the hydrogen bond network rearrangements responsible for water's anomalies[1097].

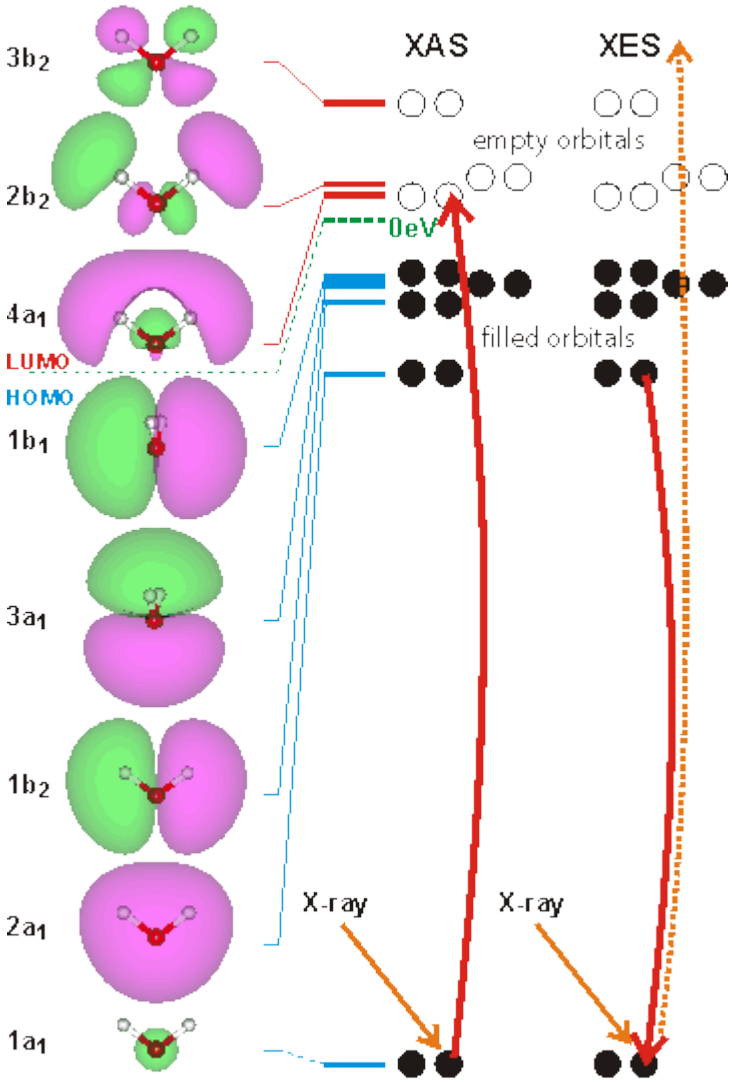
Terahertz spectroscopy and two-dimensional Raman-THz spectroscopy [2050](1 - 6 THz = 33 - 200 cm^{-1}) may be used to investigate hydrogen bonding and water dynamics on the picosecond timescale. These can also be used to investigate the hydration layers around biomolecules [1196, 1427]. This spectroscopy is powerful for examining changes in water mobility and shows changes in the water structuring at greater distances from biomolecules than those found using NMR or neutron scattering [1811]. The terahertz region is expected to be a very important region for cluster and biomolecule vibrations [1783] and provides information about the water further from the surface than NMR or diffraction methods [1368]. The hydration state of solutions may be examined using terahertz time-domain attenuated total reflection spectroscopy, which makes use of the complete complex dielectric function; both the dielectric loss, due to relaxation motion of water, and the changes in the real part of the dielectric constant [1437]. [**Back to Top** ]

X-Ray spectroscopy

X-ray-based techniques can be used to study the hydrogen-bond network of liquid water [1965]. As the techniques have an attosecond timescale, they reflect the instantaneous molecular configuration of the water molecules.



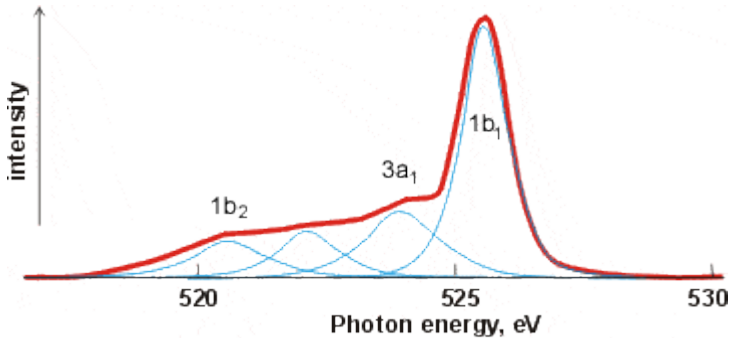
X-Ray absorption spectroscopy (XAS) involves the absorption of high-energy photons to excite core (1s, 2s) electrons to unoccupied p-character valence levels. The energies required are sensitive to the local environment mostly providing information concerning the hydrogen bond donating orbitals of the absorbing molecule at very short timescales (~ 10^{-18}s) [373, 690a, 1757]. At high photon energy, expelled electrons have sufficient energy to escape from their atom (to the continuum) and at higher energies these escaped electrons may produce signals due to backscattering. Care must be taken to avoid artifacts at the water-air surface due to desorption [1440]. X-ray Raman scattering (XRS) gives similar results to XAS and involves the inelastic scattering of x-rays from the core electrons.



Above left is shown the O K-edge (1s) XAS of liquid water (red) with possible underlying Gaussian (blue) and continuum (green) contributions.

The first peak at about 535 eV is due to excitation from the 1s orbital to the vacant O-H $4a_1$ antibonding orbital. Assignment of these gaussian peaks to electron transitions depends heavily on the underlying theory (for example, see the current dispute) as there is little structure in the experimental spectrum, this spectrum varies from run to run [690c], between methods and between different energies of the X-rays.

Thus, data fitting is error-prone. An interesting discussion of these differences has been recently published [834].



Related to XAS is the X-ray emission spectra (XES, [1757]) involving transitions from the three outermost occupiedmolecular orbitals $1b_1$, $3a_1$ and $1b_2$ to fill the vacancy (produced as above) in the $1a_1$ (1s) orbital [411]; peaks occurring in the 520-528 eV range and corresponding to instantaneous local structuring (~ 10^{-15}s). Recent X-ray emission spectroscopy shows the presence in liquid water of two well-separated peaks (split from the lone-pair non-bonding $1b_1$ peak) that interconvert but do not broaden with changes in temperature [1639]. This strongly supports a two-state network model such as presented here. [**Back to Top**]

Another recently improved X-ray technique is small-angle X-ray scattering (SAXS, [1757]) which is sensitive to density inhomogeneities in the nm-range has confirmed the existence of two distinctly different hydrogen-bonded environments in liquid water [1757].

Footnotes

Consider, for example, the very different photographs obtained at night of cars on the road between the use of fast flash and a natural light long exposure. [Back]

There is evidence, however, that the spectra may be due to a continuous variation in the hydrogen bonding and not due to discrete but overlapping populations [875]. [Back]

Gaussian curve (normal distribution curve) may be represented by the relationship:

$$A = A_{max} \times e^{-0.5 \times \left(\frac{\lambda - \lambda_{max}}{W / \left(2 \times \sqrt{2 \times Ln(2)}\right)} \right)^2}$$

where A is the amplitude, A_{max} is the peak height, λ is the wave number , λ_{max} is the wavenumber at the peak and W is the width of the peak at half height. [Back, 2]

[d] ^{16}O and ^{18}O have no nuclear spin.

Source: http://www1.lsbu.ac.uk/water/methods.html