Neutron Scattering Investigations on the Liquid-Liquid Transition in Deeply Cooled Confined Water

by

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Chairman, Department Committee on Graduate Students
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Abstract

Water is ubiquitous but mysterious. It exhibits anomalous thermodynamic behaviors at low temperatures. In addition, the glassy water, also called amorphous ice, exhibits polyamorphism. These phenomena could be understood if one accepts that a first-order low-density liquid (LDL) to high-density liquid (HDL) phase transition exists in the deeply cooled region (below 230 K) of water. However, the experimental test on the LDL and HDL in bulk water is practically difficult due to the crystallization below the homogeneous nucleation temperature (~232 K at atm). It is found that, by confining water in a hydrophilic nanoporous material, MCM-41, the homogeneous nucleation process can be avoided, which allows us to keep water in liquid state at least down to 130 K. Therefore, the confined water provides us an opportunity to detect the hypothetical HDL and LDL in the deeply cooled region of water. This thesis is devoted to the detection of the first-order LDL-to-HDL transition in the water confined in MCM-41.

In this thesis, the phase behaviors of the deeply cooled water confined in MCM-41 are investigated. With elastic neutron scattering, we measure the average density of the confined water at low temperatures and high pressures. The results show the existence of a first-order LDL-to-HDL transition in such system. The phase separation starts from 1.12±0.17 kbar and 215±1 K and extends to higher pressures and lower temperatures in the phase diagram. This starting point could be the liquid-liquid critical point of the confined water. The locus of the Widom line in the phase diagram is also determined. Parallel to the density measurement, the dynamic properties, including the so-called “boson peak”, the librational
motion, and the relaxation process of the confined water, are also studied with dynamic neutron scattering. The time scales of these motions cover a broad range from $10^{-2}$ picosecond to hundreds of nanoseconds. The results confirm the phase diagram obtained with the density measurement and show that the HDL and LDL differ in the hydrogen-bond strength and the configuration of the local hydrogen-bond network. Combining all the experimental results, we provide a clear evidence for the existence of the LDL and HDL in the confined water.

Water is crucial to protein dynamics. We investigate the role water plays in sub-picosecond collective vibration of protein with inelastic X-ray scattering and find that the hydration water makes the intra-protein longitudinal phonons “softer”. We also study the slow dynamics of the protein hydration water with quasi-elastic neutron scattering. A hydration-dependent dynamic crossover phenomenon is found.

The observation of the liquid-liquid transition in the confined water has potential to explain the mysterious behaviors of water at low temperatures. In addition, it may also have impact on other disciplines, because the confined water system represents many biological and geological systems where water resides in nanoscopic pores or in the vicinity of hydrophilic or hydrophobic surfaces.

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Chapter 1  Introduction

1.1 Anomalous Behaviors of Water and the Hypotheses

Water is the most essential and ubiquitous liquid on Earth. Leonardo da Vinci described water as "the vehicle of nature" ("vetturale di natura") because of the important role water plays in the natural sciences. People started to study water thousands of years ago. Ancient Greeks considered water, as well as earth, air and fire, as the "four elements" that everything was made up of. Similarly, ancient Chinese believed that water is one of the "five elements" with metal, wood, fire and earth.

![Figure 1.1. Comparison of the isobaric temperature dependence of the density $\rho$, thermal expansivity $\alpha$, isothermal compressibility $\kappa_T$ and isobaric heat capacity $c_p$ for water and a simple liquid [1].](image)

Water is a continuing source of fascination to scientists not only due to its tremendous political, cultural and historical significance, but also because of its anomalous physical behaviors. It is well-known that water has a density maximum at 4 °C under ambient pressure. In fact, when cooling down, water’s thermodynamic response functions and transport coefficients also exhibit counterintuitive behaviors [1-4]. Figure 1.1 shows the comparisons between some thermodynamic properties of water and those of simple...
liquids [1]. In addition, the glassy water, also called amorphous ice, exhibits polyamorphism. Experiments show that two kinds of amorphous ice, the low-density amorphous ice (LDA) and the high-density amorphous ice (HDA), exist at very low temperatures [5-7]. These two phases can transform to each other through a first-order-like transition [7, 8]. Figure 1.2 shows the HDA sample and LDA sample with the same weight [9]. One can find that their volumes, or densities, are quite different.

![Figure 1.2. Comparison of LDA (shown in panel a) and HDA (shown in panel b) with same weight [9]. The volume of LDA is larger than that of HDA, which shows that the LDA has a lower density than HDA.](image)

To account for these mysterious phenomena, several theoretical scenarios have been proposed. Currently, four scenarios are under active scrutiny [10]. They are: (1) the stability limit (SL) scenario [11]; (2) the liquid-liquid critical point (LLCP) scenario [12]; (3) the singularity-free (SF) scenario [13-15]; and (4) the critical point free (CPF) scenario [16]. Note that, the LLCP, SF and CPF scenarios hypothesize that there are two liquid phases exist in the deeply supercooled region of water: low-density liquid (LDL) phase and high-density liquid (HDL) phase. Figure 1.3 shows the schematic phase diagram of the LLCP scenario [4]. It can be found that, in this scenario, the LDL and HDL phases are thermodynamic extensions of the LDA and HDA phases into the liquid state, respectively. The transition between LDL and HDL is a first-order phase transition. This liquid-liquid phase transition ends at a critical point, which
is called liquid-liquid critical point (LLCP). The Widom line is the extension of the liquid-liquid transition line into the one-phase region. It is the locus of the maximum of correlation length [17].

Figure 1.3. The phase diagram of the LLCP scenario [4]. C and C' denote the known liquid-gas critical point and the hypothesized liquid-liquid critical point respectively. F denotes the line of first-order phase transitions that emanates from C' and separates the high-density and low-density phases that occur for temperatures below $T_c$. $W$ denotes the Widom line. The curves denoted $L$ and $H$ are the limits of the metastability of the HDA and LDA phases, respectively. $T_{H}$ denotes the homogeneous nucleation temperature line. $T_{X}$ denotes the crystallization temperatures of amorphous ice.

Theoretical and computer simulation studies strongly suggest the existence of LDL and HDL. Therefore, the detection of the LDL and HDL phases in liquid water is crucial to understand the anomalous behaviors of water. This is the motivation of this thesis.
1.2 Why Confinement?

The melting point of water at ambient pressure is about 273 K. However, with careful cooling, water can be supercooled to temperatures below 273 K. The limit of this supercooling procedure is about 232 K at ambient pressure [1]. Below this temperature, the homogeneous nucleation process cannot be avoided and liquid water will transform to crystalline phase inevitably. This temperature is called homogeneous nucleation temperature ($T_{nh}$). At very low temperatures, water exists as amorphous ice. Heating the amorphous ice will lead to a transition to crystalline phases when crossing the crystallization temperature of amorphous ice ($T_X$). Therefore, between $T_X$ and $T_{nh}$, bulk water cannot stay in liquid state. For this reason, H. E. Stanley and his collaborators called the region between $T_X$ and $T_{nh}$ “no man’s land” [12].

Unfortunately, the hypothetical LDL and HDL phases and the LLCP are in the “no man’s land” [12]. Thus the experimental detections of the liquid-liquid transition and its critical point in bulk water are almost impossible. In order to enter the “no man’s land”, in this thesis project, a hydrophilic nanoporous silica material, MCM-41, is used to confine the water. Such “strong confinement” can suppress the homogeneous nucleation process, so that it can keep the confined water in liquid state at temperatures even below $T_{nh}$.

MCM-41 is a kind of mesoporous silica material. It is made by calcining self-assembled micellar templated silica matrices, which are consisted of grains at the order of micrometer size. In each grain, parallel and uniform sized cylindrical pores are arranged in a well-ordered two-dimensional hexagonal lattice. MCM-41 has hydrophilic surface and large pore volume to confine sufficient amount of water, and also small enough pore size to inhibit the homogeneous nucleation temperature of water. From a series of differential scanning calorimeter (DSC) measurements, we confirmed that when the nominal pore size is smaller than $\sim 17$ Å, the ice nucleation can be bypassed and the confined water can be supercooled at least down to $\sim 130$ K without freezing [18].

Figure 1.4 shows the two-dimensional hexagonal geometry of pores in MCM-41. Distances “a” and “d” represent the inter-pore and inter-plane distances respectively.
Figure 1.4. Two-dimensional hexagonal pore structure of MCM-41 used in this thesis project. The center-to-center distance between two adjacent pores is marked as "a", and the distance between two adjacent rows is marked as "d". Notice that \( d = \sqrt{3}a/2 \), which equals to 29 Å.

In this experiment, MCM-41 with nominal pore size of 15 Å was used as the confining matrix. The pore size is estimated by Barret-Joyner-Halenda (BJH) method with nitrogen sorption isotherms [19, 20]. Water can be introduced into the pores via vapor condensation easily. The full hydration level by weight \( h \) (g water/g dry MCM-41) is about 0.45 g/g for H₂O-hydrated sample, and 0.50 g/g for D₂O-hydrated sample. The adsorption isotherm data presented in Fig. 1.5 justifies these designations.

![Diagram of MCM-41 pore structure](image)

Figure 1.5. Isothermal adsorption of water vapor (H₂O) onto MCM-41 at room temperature. \( P_0 \) denotes the ambient pressure. The horizontal line marks the full hydration level of the sample.

Confining water with MCM-41 provides a chance to detect the liquid-liquid transition and its critical point in the deeply supercooled water. Note that, the restricted geometry and the water-surface
interactions are influential to the properties of the confined water. Therefore, to what extent the confined water is similar to the bulk water is still in debate [21-23]. However, such a confined water system is of fundamental importance in practice and fascinates scientists from different disciplines. For example, it represents many biological and geological systems where water resides in nanoscopic pores or in the vicinity of hydrophilic or hydrophobic surfaces.

1.3 Previous Results

In the past ten years, a group led by Prof. S.-H. Chen at Department of Nuclear Science and Engineering, Massachusetts Institute of Technology performed extensive studies on the water confined in MCM-41 with neutron scattering technique. In this section, I list some important observations relevant to the confined water system. Their relations to the hypothetical liquid-liquid transition will be discussed in the following parts of this thesis.

In 2004, Faraone et al. observed a dynamic crossover phenomenon at about 225 K in water confined in the MCM-41 [24] with quasi-elastic neutron scattering (QENS). The existence of this phenomenon was confirmed by nuclear magnetic resonance technique [25]. The dynamic crossover refers to the phenomenon that the temperature dependence of the characteristic relaxation time of water transforms from a “fragile” behavior at high temperatures to a “strong” behavior at low temperatures. The “fragile” behavior of the characteristic relaxation time is liquid-like, and can be described by the Vogel-Fulcher-Tammann (VFT) law:

\[
\langle \tau \rangle \propto \exp\left[\frac{D(T_0 - T)}{T - T_0}\right]
\]

(1.1)

where \(\langle \tau \rangle\) is average characteristic relaxation time of the system. \(D\) is a constant providing the measure of fragility. \(T_0\) is the ideal glass transition temperature. The “strong” behavior of the characteristic relaxation time is solid-like, and can be described by the Arrhenius law:

\[
\langle \tau \rangle \propto \exp\left(\frac{E_A}{k_B T}\right)
\]

(1.2)

where \(E_A\) is the activation energy, \(k_B\) is the Boltzmann constant.
The temperature at which these two behaviors intersect is called crossover temperature \((T_c)\). Two typical dynamic crossover phenomena in the water confined in the MCM-41 are shown in Fig. 1.6.

![Figure 1.6. Dynamic crossover phenomena in the water confined in MCM-41 with pore diameter of 18 Å (a) and 14 Å (b) [24]. The solid lines are the fit to the VFT law. The dotted lines are the fit to the Arrhenius law. \(T_c\) is the crossover temperature at which the two laws intersect.](image)

The dynamic crossover is similar to the "fragile-to-strong transition" proposed by Ito et al. [26]. This "fragile-to-strong transition" is described as follows. At high temperatures, water behaves like liquid. The temperature dependence of the structural relaxation is "fragile" and can be described by the VFT law. As cooling down and approaching the ideal glass transition temperature \(T_0\), the structural relaxation time of water increases rapidly. Nevertheless, before arriving at \(T_0\), the activated hopping mechanism will dominate the structural relaxation, and the temperature dependence of the structural relaxation transforms to a "strong" behavior that can be described by the Arrhenius law. By this "fragile-to-strong transition" mechanism, the dynamic arrest transition, which is predicted by ideal mode-coupling theory [27], can be avoided.
Inspired by the "fragile-to-strong transition" proposed by Ito et al., S.-H. Chen and his collaborators named the dynamic crossover "fragile-to-strong crossover". They ascribed its origin to the change of the local structure of the confined water as crossing the crossover temperature [28]. At temperatures higher than $T_x$, the local structure of the confined water is HDL-like and the relaxation exhibits "fragile" behavior. While at temperatures lower than $T_x$, the local structure of the confined water is LDL-like. The local structure of LDL is very similar to that of the ice I_h [29], thus the hopping process dominates the structural relaxation, which leads to a "strong" behavior of the relaxation.

Xu et al. relate the "fragile-to-strong crossover" in water to crossing the Widom line of the liquid-liquid transition with computer simulation study [30]. They find that the crossover temperature coincides with the Widom line temperature. Therefore, by detecting the dynamic crossover one can determine the Widom line of the liquid-liquid transition. Liu et al. measure the dynamic crossover at high pressures with QENS [31]. They find that the crossover temperature decreases as pressure increases below about 1.6 kbar. Above 1.6 kbar, the dynamic crossover cannot be identified anymore. They ascribe the dynamic crossover observed at pressures lower than 1.6 kbar to the crossing of the Widom line, and the disappearance of the dynamic crossover at pressures higher than 1.6 kbar to the appearance of the LDL-HDL phase separation. With this logic, they find the liquid-liquid critical point, at where the Widom line and the liquid-liquid transition line intersect, locate at about 1.6 kbar and 203 K.

In 2009, Zhang et al. observed a density hysteresis phenomenon in the confined heavy water [32]. These authors measure the average density of the confined heavy water with isobaric cooling scan and warming scan by elastic neutron scattering. They find that the density profiles as a function of temperature obtained by cooling scan and warming scan are different. A typical density hysteresis is shown in Fig. 1.7. Zhang et al. perform such measurements in the pressure range from 1 bar to 2.9 kbar. It shows that the hysteresis phenomenon appears at all the measured pressures. The amplitude of the hysteresis increases as the pressure increases from 1 bar to 1.5 kbar. Above 1.5 kbar, the amplitude of the hysteresis does not show effective change as pressure changes.
A first-order phase transition may exhibit metastability. Therefore, the observation of the density hysteresis strongly suggests the existence of the LDL and HDL phases and the liquid-liquid transition in the confined water.

![Figure 1.7](image)

Figure 1.7. A typical density hysteresis in the confined heavy water [32]. Upper panel shows the density profiles as a function of temperature obtained with warming scan (red circles) and cooling scan (black squares) at 2.5 kbar. A significant hysteresis can be found between these two profiles. Lower panel shows the difference between the density profile obtained with cooling scan and that obtained with warming scan.

In addition, Chen’s group find a broad peak emerges in the low-frequency region of the inelastic neutron scattering (INS) spectrum of the confined water when the temperature is lower than about 230 K at ambient pressure [33]. This peak is usually called “boson peak”. Boson peak is a universal feature for all the disordered materials. Figure 1.8 shows the emergence of the boson peak in the INS spectrum of the confined water.

Notice that, the temperature of the emergence of the boson peak, 230 K, is quite similar to the crossover temperature. Therefore, the authors tentatively ascribe the emergence of the boson peak to the crossing of the Widom line from above.
Figure 1.8. Boson peak in the confined water at ambient pressure \[33\]. It can be found that boson peak becomes visible only when the temperature is lower than about 230 K.

Figure 1.9. Unusual phenomena observed in the confined water in the \(P-T\) plane (till 2011). Black squares denote the positions of the dynamic crossover in confined H\(_2\)O \[31\]. Black pentacle denotes the LLCP in confined H\(_2\)O estimated by Liu et al. \[31\]. Dashed line denotes the liquid-liquid transition line in confined H\(_2\)O estimated by Liu et al. \[31\]. Red circles denote the positions of the density hysteresis in confined D\(_2\)O. Blue triangle denotes the position of the emergence of the boson peak in confined H\(_2\)O. Green line denotes the LDA-HDA coexistence line in bulk water.
The loci of the above-mentioned phenomena in the pressure-temperature plane are shown in Fig. 1.9. Generally, all these phenomena can indicate some kinds of transitions. Notice that, these results are not coherent. Particularly, the positions of the density hysteresis [32], which denote the liquid-liquid transition line, are very different from the liquid-liquid transition line estimated by Liu et al. [31]. Another point is that, the dynamic crossover disappears at pressures higher than 1.6 kbar, however, the density hysteresis exists at all the measured pressures up to 3 kbar. Thus, till the year of 2011, even many interesting phenomena had been observed, a coherent picture for the phase behavior of the deeply supercooled confined water still lacked. In the past three years, we investigated these phenomena extensively, and obtained a clearer picture for the phase diagram of the deeply supercooled confined water.

1.4 Survey of the Thesis

This thesis summarizes my research work during the past five years at MIT. The aim of my research is to figure out the phase behavior of the deeply supercooled water confined in MCM-41. I first approach this problem by clarify the phase diagram of the confined water system, including the liquid-liquid transition line, the Widom line and the LLCP, with elastic neutron scattering (Chapter 2). In Chapter 3, I verify the phase diagram obtained in Chapter 2 by measuring the dynamic properties of the confined water system. In addition, it is well-known that the surface water is essential to the biological function of proteins. In Chapter 4, I investigate the dynamics of the protein-water system in both short time scale and long time scale.
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Chapter 2  Detection of the Liquid-Liquid Transition: Density Measurement

2.1 Model Description

The order parameter of the hypothetical liquid-liquid transition is density. Therefore, it is crucial to know how to measure the average density of the water confined in MCM-41. In 2007, Liu et al. developed a method for the measurement of the average density of the confined heavy water with small angle neutron scattering technique [34]. In this section I will introduce this method first.

As shown in Fig. 1.4, the two-dimensional structure of MCM-41 has a hexagonal order. This order will produce a Bragg peak in the neutron diffraction spectrum of the confined water system. The center of the Bragg peak locates at $Q = 2\pi/d = 0.21 \text{ Å}^{-1}$. A typical neutron diffraction spectrum of the confined water system is shown in Fig. 2.1.

![Figure 2.1](image)

Figure 2.1. Typical neutron diffraction spectrum of the confined heavy water system (denoted by black solid squares) at 1 bar and 295 K. The red curve is the fitting curve with Eq. 2.2. The green dashed line represents the “effective” part of the spectrum, i.e., the first term in the right-hand-side part of Eq. 2.2. The dash-dot line represents the background, i.e., the terms $BQ^{-\beta} + C$ in Eq. 2.2.
The measured neutron diffraction spectrum of the confined heavy water system consists of three parts: (i) the low-\(Q\) scattering of the fractal packing of the MCM-41 grains and the background signal due to the low-\(Q\) nature of the instrument; (ii) the \(Q\)-independent incoherent background and (iii) a Bragg peak at \(2\pi/d\) due to the two-dimensional hexagonal lattice of the MCM-41. The first part is represented by \(BQ^\beta\). The second part is represented by a constant \(C\). The third part is given by \(nV_p^2(\Delta \rho_{\text{std}})^2\tilde{P}(Q)S(Q)\), where \(n\) is the number of scattering units (water cylinders) per unit volume, \(V_p\) is the volume of the scattering unit, \(\Delta \rho_{\text{std}} = \rho_{\text{D}_2\text{O}} - \rho_{\text{MCM}}\) is the difference of the scattering length density (sld) between the scattering unit (D\(_2\)O cylinder) and the environment (MCM-41). \(\tilde{P}(Q)\) is the form factor of the scattering unit. \(S(Q)\) is the inter-cylinder structure factor of the two-dimensional hexagonal lattice. The sld of the scattering unit (D\(_2\)O cylinder) \(\rho_{\text{D}_2\text{O}}\) is proportional to the average density of the confined D\(_2\)O \(\rho_{\text{D}_2\text{O}}\): 
\[
\rho_{\text{D}_2\text{O}} = \alpha nA_{\text{bulk}}/M,
\]
where \(\alpha = N_{\text{A}}\Sigma b_i/M\), \(N_{\text{A}}\) is Avogadro’s number, \(M\) is the molecular weight of D\(_2\)O and \(b_i\) is the coherent scattering length of the \(i\)th atom in the scattering unit. The form factor \(\tilde{P}(Q)\) for a long cylinder (\(QL > 2\pi\)) is given by \((\pi/QL)(2J_1(QR)/QR)^2\), where \(R\) is the radius of the cylinder, \(L\) is the length of the cylinder and \(J_1(x)\) is the first-order Bessel function of the first kind. The inter-cylinder structure factor of the two-dimensional hexagonal lattice \(S(Q)\) could be modeled by a Lorentzian function. Therefore the \(Q\) scan intensity distribution of the system can be modeled as:
\[
I(Q) = nV_p^2(\alpha \rho_{\text{D}_2\text{O}}^m - \rho_{\text{MCM}}\rho_{\text{D}_2\text{O}})^2\frac{\pi}{QL} \left[\frac{2J_1(QR)}{QR}\right]^2 \left[\frac{\frac{1}{1}\Gamma}{\left(Q - \frac{\pi^2}{d}\right) + \left(\frac{1}{2}\right)^2}\right] + BQ^\beta + C.
\]
(2.1)

Eq. 2.1 can be rewritten as:
\[
I(Q) = A\frac{J_1(QR)^2}{Q^3R^2} \left[\frac{\frac{1}{1}\Gamma}{\left(Q - \frac{\pi^2}{d}\right) + \left(\frac{1}{2}\right)^2}\right] + BQ^\beta + C
\]
(2.2)

where \(A\) is expressed as:
\[
A = nV_p^2\alpha^2 \left(\rho_{\text{D}_2\text{O}}^m - \rho_{\text{MCM}}\rho_{\text{D}_2\text{O}}\right)^2 \frac{4\pi}{L}.
\]
(2.3)
Notice that the average density of the confined D\textsubscript{2}O \(\rho_{\text{D}2\text{O}}\) is contained in \(A\). The value of \(A\) can be obtained by convoluting Eq. 2.2 with the instrument resolution and fitting it to the measured \(Q\) scan data. Figure 2.1 shows the fitting curve using this model. In order to determine \(\rho_{\text{D}2\text{O}}\) from \(A\), one needs to know the value of the constant \(A_1\), which can be found with the following method. According to a previous study [35], the value of \(\rho_{\text{D}2\text{O}}\) at \(T = 290\) K under ambient pressure is 1.175±0.005 g/cm\(^3\). Under this circumstance, \(A\) can be obtained by fitting. Meanwhile, \(\rho_{\text{MCM}}^\text{std}\) and \(\alpha\) can be obtained by contrast variation [34]. Thus the value of the constant \(A_1\) can be determined through Eq. 2.3.

In Eq. 2.2, \(R, B, C\) and \(\beta\) are constants. The value of \(d\), which reflects the structure of the MCM-41, depends on temperature and pressure very weakly. This is because (i) the thermal expansion coefficient of the MCM-41 is only in the order of \(10^{-6}/\text{K}\), which is smaller than that of the water by three orders; (ii) as a solid, the MCM-41 exhibits very small compressibility. Considering that \(d\) is almost a constant, one can find that at \(Q = 0.21\) Å\(^{-1}\) (we denote this \(Q\) value as \(Q_B\) in the following part since it is close to the position of the Bragg peak \(2\pi/d\)) the \(Q\) scan intensity is expressed as:

\[
I(Q_B) = nV_p^2 \left( \alpha \rho_{\text{D}2\text{O}}^m - \rho_{\text{MCM}}^\text{std} \right)^2 \frac{\pi}{Q_B L} \left[ \frac{2J_1(Q_B R)}{Q_B R} \right]^2 \left[ \frac{1}{2} \frac{1}{(Q_B \frac{2\pi}{d} + (\frac{1}{2} \Gamma)^2)} \right] + BQ_B^{-\beta} + C
\]

\[
\approx nV_p^2 \left( \frac{2J_1(Q_B R)}{Q_B R} \right)^2 \frac{\pi}{\Gamma} \alpha^2 \left( \rho_{\text{D}2\text{O}}^m - \frac{\rho_{\text{MCM}}^\text{std}}{\alpha} \right)^2 + BQ_B^{-\beta} + C
\]

\[
= D \left( \rho_{\text{D}2\text{O}}^m - \frac{\rho_{\text{MCM}}^\text{std}}{\alpha} \right)^2 + BQ_B^{-\beta} + C
\]

where

\[
D = nV_p^2 \frac{\pi}{Q_B L} \left[ \frac{2J_1(Q_B R)}{Q_B R} \right]^2 \frac{\pi}{\Gamma} \alpha^2.
\]

In Eq. 2.5, \(\Gamma\) exhibits weak temperature and pressure dependences. Subsequently, the value of \(D\) can be considered approximately as a constant in certain pressure and temperature ranges. Therefore, from the last step of Eq. 2.4 one can find that \(I(Q_B)\) is a monotonic function of \(\rho_{\text{D}2\text{O}}^m\). Knowing this, one can use \(I(Q_B)\) to monitor the change of \(\rho_{\text{D}2\text{O}}^m\).

Liu et al. use this model to study the average density of the confined heavy water as a function of temperature at ambient pressure [34]. They find a well-defined minimum point at 210 K, which is
consistent with the computer simulation prediction [36]. The density profile of the confined heavy water is shown in Fig. 2.2.

![Density profile](image)

**Figure 2.2.** Average density of the confined D\textsubscript{2}O at ambient pressure. A smooth transition of D\textsubscript{2}O density from the maximum value at 284 K to the minimum value at 210 K is clearly shown. The filled squares are the density data for bulk D\textsubscript{2}O taken from the CRC Handbook [37].

The observation of the density minimum is significant. The density minima in liquids are even rarer than density maxima. Confirming the existence of a density minimum in water would reveal much about the supercooled state of this important liquid. Its occurrence would signal the reversal of the anomalies that set in near the density maximum; *i.e.*, that mildly supercooled water is anomalous but that deeply supercooled water "goes normal" [34]. In addition, the existence of the density minimum in water is ascribed to the local tetrahedral structure, and is consistent with the existence of the liquid-liquid phase transition [36].
2.2 Liquid-Liquid Transition in Confined Water

The existence of the density minimum in confined water is consistent with the liquid-liquid transition hypothesis. However, it does not provide any direct proof for the hypothetical first-order liquid-liquid transition.

It is common that the first-order phase transition exhibits metastability. Therefore, one can test the existence of the hypothetical first-order liquid-liquid transition by detecting the hysteresis of the order parameter, the density of water. In 2011, Zhang et al. attempted to detect the hypothetical liquid-liquid transition in the heavy water confined in MCM-41 [32]. They measured the average density of the confined D$_2$O with warming and cooling scans at pressures from 1 bar to 2.9 kbar. The main result is shown in Fig. 2.3 (The data at 3.3 k and 4 kbar in Fig. 2.3 were measured with a similar method [38]), and can be summarized as follows. (1) Density hysteresis phenomenon is observed at all the measured pressures below ~3.5 kbar. (2) When the pressure is below ~1.5 kbar, the hysteresis enhances as the pressure increases. The maximum density differences between the cooling and warming scans are 0.01 g/cm$^3$ at 1 bar, 0.017 g/cm$^3$ at 1 kbar, and 0.031 g/cm$^3$ at 1.5 kbar, respectively. (3) When the pressure is above ~1.5 kbar, the amplitude of the hysteresis stabilizes at about 0.03 g/cm$^3$. (4) The temperature of the maximum density difference between the cooling and warming scans shifts to lower temperature as the pressure increases. Zhang et al. attribute this strong hysteresis phenomena observed at pressures higher than ~1500 bar to the crossing of the liquid-liquid transition line, due to the discontinuity at the phase boundary and the strong metastability of the liquid water in the coexisting region [39] as the result of the liquid-liquid transition [40, 41] and to the confinement [42, 43]. The hysteresis observed below ~1000 bar, which are relatively weak, are attributed to possible temperature lags between the warming and cooling scans rather than to the crossing of a phase boundary. However, this conclusion was soon challenged by Limmer and Chandler [44]. With a computer simulation study employing mW model of water, these researchers attribute all of the observed density hysteresis phenomena to a liquid-solid transition (LST) in the confined water (this result is also in debate [45, 46]). An important difference between the LLCP
scenario and LST scenario is that in the LLCP scenario there is a LLCP that terminates the liquid-liquid transition line at a positive pressure. In contrast, in the LST scenario there is no associated critical point and the liquid-solid transition line exists in all the positive pressures.

Figure 2.3. The density measurement on the confined D$_2$O made by Zhang et al. (1 bar to 2.9 kbar) [32] and Wang et al. (3.3 k and 4 kbar) [38]. (a) The density profiles of confined D$_2$O with warming and cooling scans at different pressures. The data are shifted by 0.05 g/cm$^3$ between adjacent pressures for clarity. (b) The density differences between the cooling and warming scans at different pressures. The data are shifted by 0.03 g/cm$^3$ between adjacent pressures for clarity.

In order to clarify the nature of the transition in the deeply-cooled confined D$_2$O, we performed a series of neutron diffraction experiments to measure the average density of the D$_2$O confined in MCM-41 with warming and cooling scans at different pressures [47]. The experiments were performed at the cold neutron spin polarized inelastic neutron spectrometer (SPINS) and a small-angle neutron scattering
instrument (SANS) at National Institute of Standards and Technology Center for Neutron Research. The full hydration level of the sample is \( h = 0.5 \) g/g. The method to extract the average density of the confined water from the neutron diffraction spectra is described in Section 2.1. In the study of Zhang et al. [32], the researchers performed the temperature scans with the following procedure: for each pressure, the sample was cooled from 300 K to 130 K at ambient pressure and then pressurized to the desired value. After 2 hours of waiting, the warming scan with 0.2 K/min was first performed from 130 K to 300 K. When the warming scan was finished, they waited for another 2 hours and then performed the cooling scan with 0.2 K/min from 300 to 130 K (the data at 3.3 and 4 kbar in Fig. 2.3 are measured by similar protocol but with cooling scan first [38]). Note that, in such continuous temperature scans, the temperature changes continuously with constant speed. Though the speed is slow, it is possible that the heat transfer does not complete and the temperature sensor, which is on the aluminum holder of the sample, cannot accurately reflect the temperature of the confined water. In this case, there may be a temperature lag between the warming and cooling scans, and a hysteresis that is not due to the phase transition may appear. To eliminate the possible temperature lag, in this study, we use a new protocol for the temperature scan. For each pressure, we only measure several important temperatures around which the density hysteresis takes place. In addition, before each measurement, we wait for half an hour after the temperature reaches the desired value. Therefore, there is sufficient time for the sample to get a uniform temperature distribution and to reach temperature equivalence to the sample holder. The result of the density measurement with this new protocol is shown in Fig. 2.4. It is found that the effective density hysteresis only appears when the pressure is higher than about 1500 bar. It takes place at the temperature that is very close to the one found in Ref. [32]. This result suggests a first-order transition between a low-density phase and a high-density phase, and is consistent with the LLCP picture, rather than the LST picture. The end point of the phase separation, which locates at 1.29±0.34 kbar and 213±3 K, is the LLCP of the confined D\(_2\)O according to the LLCP scenario. In previous studies [31, 48], the researchers estimated the critical pressure of the confined H\(_2\)O to be 1.5±0.3 kbar by the dynamical properties of the system. The critical pressure obtained here agrees with the previous estimations on the confined H\(_2\)O fairly well. Furthermore,
above the critical pressure, the maximum density difference increases as the pressure increases (0.010±0.003 g/cm³ at ~1.6 kbar; 0.016±0.003 g/cm³ at ~2.5 kbar), which agrees with an idea that the phase separation becomes more significant as the distance from the critical point increases along the liquid-liquid transition line.

Figure 2.4. The density measurement on the confined D₂O with the new temperature scan protocol. The left column shows the density profiles with warming and cooling scans at $P \sim 2500$ bar (a1), 1600 bar (a2), 1000 bar (a3) and 1 bar (a4). The right column shows the density differences between the cooling and warming scans (denoted by black circles) at $P \sim 2500$ bar (b1), 1600 bar (b2), 1000 bar (b3) and 1 bar (b4). We also plot the results of the density differences in Ref. [32] (denoted by red circles) for comparison. The dashed lines are drawn to guide eyes.

We also tried other waiting times from 25 to 50 minutes for the density measurements at ~1.6 kbar. The result shows that the value of the average density of the confined D₂O is effectively constant for
different waiting times used here. This observation suggests that after waiting for 25 minutes, the sample temperature becomes stable and no evident transition happens up to 50 minutes.

In this section, we confirm the existence of the density hysteresis at the pressures higher than 1.5 kbar. These hysteresis phenomena suggest the existence of the liquid-liquid transition line in the confined water with its end point at $1.29 \pm 0.34$ kbar and $213 \pm 3$ K. I will discuss the nature of this end point and the Widom line in next section.

2.3 Widom Line, Liquid-Liquid Critical Point and Phase Diagram

From Fig. 2.4 one can find that, below about 1000 bar, no effective hysteresis is observed. This result is different from the result in Ref. [32]. Such difference could be due to the temperature lag between the warming and cooling scans in the previous study. In principle, the influence of the temperature lag on the density measurement has a positive correlation with the isobaric heat capacity of the confined water ($C_p$). Therefore, the hysteresis at low pressures may indicate the maximum of $C_p$. This conjecture can be justified as follows. According to relevant thermodynamic studies [49, 50], at ambient pressure, the peak position of $C_p$ of the D$_2$O confined in MCM-41 with the pore diameter of 17 Å is 240 K, which is very close to the temperature at which the maximum density difference takes place at ambient pressure in Ref. [32], 243 K (see Fig. 2.4 (b4)). The small difference between these two temperatures may be due to the difference of the pore diameter. Notice that, the peak position of $C_p$ of the H$_2$O confined in MCM-41 is at 241 K with the pore diameter of 15 Å, and at 237 K with the pore diameter of 17 Å. Therefore it seems that a 2-Å difference in pore diameter can change the temperature of the peak of $C_p$ by several kelvins. Keep this idea in mind, one can then estimate the Widom line of the liquid-liquid transition, which is defined as the locus of the $C_p$ maxima in the corresponding one-phase region [30], with the position of the maximum hysteresis observed at pressures lower than the critical pressure in Ref. [32]. Note that, in many other literatures, the Widom line is defined as the locus of the maximum correlation length [17, 51]. This definition can avoid the confusion introduced by the existence of
multiple local maxima in the heat capacity of water [52]. However, in this study, we still employ the former definition, since the heat capacity of the confined water is available and thus it is easy to compare our result to the result of thermodynamic measurement. In addition, as approaching the critical point, the maximum of heat capacity and the maximum of correlation length emerge [17, 51].

![Phase diagram of the liquid-liquid transition of the confined heavy water.](image)

Figure 2.5. Phase diagram of the liquid-liquid transition of the confined heavy water. The black solid squares and the red open squares denote the positions of the maximum density differences obtained by the continuous temperature scans at pressures higher than the critical pressure and lower than the critical pressure, respectively [32, 38]. The former are due to the phase transition in the confined water, and represent the liquid-liquid transition line (denoted by a black solid line), while the latter are due to temperature lags, and represent the Widom line (denoted by a red dashed line). These two lines intersect at the LLCP, whose approximate position is denoted by an elliptical region.

Considering all the above discussions, we plot the phase diagram of the liquid-liquid transition of the confined heavy water in Fig. 2.5. The black solid squares denote the positions of the maximum density differences obtained by the continuous temperature scans at pressures higher than the critical pressure [32, 38]. These hysteresis phenomena cannot be completely eliminated by the new temperature scan protocol introduced here and denote the liquid-liquid transition of the confined water. By connecting these black solid squares with a smooth curve, and noting that the hysteresis disappears at pressures higher than 3500
bar in the temperature range from 140 to 300 K \cite{38}, we obtain the liquid-liquid transition line. The red open squares denote the positions of the maximum density differences obtained by the continuous temperature scans at pressures lower than the critical pressure \cite{32}. These hysteresis phenomena can be eliminated by the new protocol and denote the positions of the $C_P$ maximum, \textit{i.e.} the Widom line. The liquid-liquid transition line and the Widom line intersect at \(1.29 \pm 0.34\) kbar and \(213 \pm 3\) K. This point could be the LLCP according to the LLCP scenario.

It is believed that water undergoes glass transition at low temperatures \cite{53, 54}. The transition temperature $T_g$ is conjectured to be between 136 \cite{55-57} or 165 K \cite{58} for bulk water, and 165 K for the water confined in MCM-41 at ambient pressure \cite{49}. All of these temperatures are much lower than the temperatures at which the hysteresis phenomena take place. Thus the hysteresis should not be directly induced by the possible glass transition in the confined water. Another concern is that due to the possible existence of the glass transition, below the conjectured $T_g$ the confined water may be in a glassy state, rather than an (metastable) equilibrium state, and the density measurement may be affected. In order to clarify this point, we perform a warming scan on density at 2 kbar by the following steps: first cool the system to 170 K at ambient pressure, then pressurize the system to 2 kbar and start the warming scan. In this route, the system temperature keeps on higher than the conjectured $T_g$ of the confined water and the system should be always away from a glassy state. This experimental route gives an effectively same density profile as compared to the one obtained by the warming scan starting from 140 K. Therefore we conclude that the hysteresis observed in this study is not affected by the possible glass transition in the confined water.

In order to examine the obtained phase diagram and to get a general idea on how the density of the confined water behaves as a function of $T$ and $P$, we perform isobaric density measurements on the confined D$_2$O at 5 pressures: 0.1 k, 1 k, 2.5 k, 4 k and 5 kbar. The data at 2.5 kbar are measured with warming scan. The results are shown in Fig. 2.6. According to the phase diagram shown in Fig. 2.5, below \(\sim 190\) K, the former 3 pressures are in the LDL phase, while the last 2 pressures are in the HDL phase. Figure 2.6 clearly shows that below 190 K, there is an evident density gap of \(\sim 0.04\) g/cm$^3$ between
the density profiles at 0.1 k, 1 k, 2.5 kbar and the density profile at 4 kbar. This gap shows the phase separation between LDL and HDL. In this temperature range, the three density curves representing LDL phase are close to each other, which shows that the isothermal compressibility ($\chi_T$) of the LDL phase is small. At 170 K, the density only changes by $\sim0.004$ g/cm$^3$ as pressure increases from 100 bar to 2.5 kbar. In contrast, in HDL phase, the density changes by $\sim0.02$ g/cm$^3$ as pressure increases from 4 k to 5 kbar, which suggests a significantly larger $\chi_T$. The huge difference of $\chi_T$ in LDL and HDL is due to the different local structures of LDL and HDL. The LDL has a tetrahedral hydrogen-bond structure extending to the second coordination shell. While for the HDL, the second coordination shell collapses [29]. These features make the LDL more rigid than the HDL. Such sharp distinction on $\chi_T$ fades out as entering the one-phase region, which suggests that the LDL and HDL phases mix in this region.

![Graph showing density profiles for LDL and HDL phases](image)

Figure 2.6. The average density of the confined D$_2$O as a function of $T$ at $P = 0.1$ k (black squares), 1 k (red circles), 2.5 k (heating scan, blue up triangles), 4 k (green down triangles) and 5 k (magenta left triangles) bar. The left-hand-side region of the dashed vertical line is the two-phase region with its phase separation between 3 and 4 kbar. The right-hand side region of the dotted vertical line is the one-phase region.

Though the confined water system behaves differently from the bulk water due to the strong confinement, it is still interesting to compare our result to the theoretical and numerical predictions related
to bulk water. As mentioned in Chapter 1, four scenarios have been proposed for the low-temperature phase behavior of liquid water, they are (1) the stability limit (SL) scenario [11]; (2) the liquid-liquid critical point (LLCP) scenario [12]; (3) the singularity-free (SF) scenario [13-15]; and (4) the critical-point free (CPF) scenario [16]. The phase diagram obtained here is qualitatively similar to the phase diagrams suggested by LLCP scenario and SF scenario. In this study, we define the end point of the liquid-liquid transition as the LLCP of the confined heavy water. The SF scenario suggests that no singularity at the end point of the liquid-liquid transition [13]. To directly distinguish between these two scenarios one may want to study the critical behavior of this end point. Nevertheless, the quasi-one-dimensional geometry in MCM-41 can strongly suppress any critical behavior [42]. Thus to measure the critical behaviors near the end point of the liquid-liquid transition is almost impossible. In fact, as the pressure approaching the critical pressure, the absolute value of the isobaric thermal expansion coefficient $|\alpha_r|$ of the confined D$_2$O exhibits no critical phenomenon [59]. Kumar et al. suggests another method to distinguish between these two scenarios: in the LLCP scenario the maximum of $C_P$ increases with the increase of pressure, while in the SF scenario the maximum of $C_P$ does not [51]. In Ref. [32], below the critical pressure, the maximum density difference increases from 0.010 g/cm$^3$ at 1 bar to 0.017 g/cm$^3$ at ~1 kbar. Considering that the $|\alpha_r|$ increases only by 2.7% as $P$ increases from 1 bar to ~1 kbar [59], we conjecture that such big increases on maximum density difference as $P$ increases from 1 bar to ~1 kbar is mainly due to the enhancement of the temperature lag, which indicates a larger $C_P$. Following this logic, we suggest that the LLCP scenario provides a better explanation. It is worth mention that, for bulk water, recent experimental and theoretical studies support the LLCP scenario rather than the SF scenario [60-62].

2.4 Absence of the Liquid-Liquid Transition in a Partially-Hydrated Sample

Previous study [35] shows that at ambient pressure, for an 85% partially-hydrated sample, the density minimum obscures and the maximum of the absolute value of the isobaric thermal expansion
coefficient ($|\alpha|$) decreases as compared to the fully-hydrated sample. Therefore, it is interesting to examine if a reduction of $h$ can mitigate the phase transition at high pressures.

Figure 2.7. The density profiles of confined D$_2$O with warming (red up triangles) and cooling (black down triangles) scans for a partially-hydrated sample at $P \sim 1600$ bar. It is seen that no hysteresis is found in this sample. We also plot the density profiles of confined D$_2$O with warming (orange circle) and cooling (blue squares) scans for the fully-hydrated sample at $P \sim 1600$ bar for comparison.

Figure 2.7 shows the average density of the confined D$_2$O of an 80% partially-hydrated sample at $\sim 1.6$ kbar with warming and cooling scans. Strikingly, the density hysteresis completely disappears in this sample. The disappearance of the density hysteresis in the partially-hydrated sample is also observed at 1 and 2.5 kbar. Notice that, both experimental and computer simulation studies show that the confined water has layer structure [22, 63, 64]. According to Gallo et al. [22], the water confined in MCM-41 can be divided into two dynamically distinct parts in radial direction: bound water and free water. The bound water is a 3-Å-thick shell layer that coats to the hydrophilic surface of the silica cavity, while the free water is the water in the center part of the cavity. Since the water forms the shell layer first [65], the 20% lowering of $h$ is mainly due to the reduction of the free water. Thus, in this partially-hydrated sample, the amount of free water decreases by about 50% compared to its fully-hydrated counterpart. The disappearance of the density hysteresis in the partially-hydrated sample strongly suggests that (1) the free
water, not the bound water, undergoes a liquid-liquid transition, and (2) a well-developed hydrogen-bond network in free water is the necessary condition for water confined in MCM-41 to exhibit liquid-liquid transition.

2.5 Conclusion

In summary, we investigate the average density of the deeply-cooled heavy water confined in the MCM-41 over the pressure-temperature plane. By detecting the density hysteresis, we find that the transition in the system is a liquid-liquid transition, rather than a liquid-solid transition. The locus of the liquid-liquid transition line is determined. Its end point, which locates at $1.29\pm0.34$ kbar and $213\pm3$ K, could be the LLCP according to the LLCP scenario. The locus of the Widom line is also estimated. Therefore, the phase diagram of the confined water system is obtained. In addition, we measure an 80% partially-hydrated sample and it shows that no transition appears in this sample even at high pressures. This result shows that it is the free water part, rather than the bound water part, of the confined water that undergoes a liquid-liquid transition.
Chapter 3  Detection of the Liquid-Liquid Transition: Dynamic Properties

In last chapter, I introduce our attempt to establish the existence of the first-order liquid-liquid transition in the confined water with density measurement. This study is a thermodynamic measurement on the equilibrium properties of the system. The relevant measurements on dynamic properties of the confined water system, however, still lack. In fact, various dynamic properties, including structural relaxation [31, 41], stretching vibration [40, 41], mean square displacement [66], etc., have been measured to study the phase behaviors of the aqueous solutions or confined water. The examinations of the phase behaviors by the dynamic properties are indispensable, because they provide complementary insights to the thermodynamic and structural results.

In this chapter, I will introduce our measurements on various dynamic properties, including the so-called “boson peak”, the librational band of the vibrational density of state, and the slow dynamics, of the confined water system. We found that these results are intimately correlated to the thermodynamics of the confined water.

3.1 Vibrational Density of State of Condensed Matter

The beginning of this chapter goes to a review on the relation between the vibrational density of state (vDoS) of the condensed matter and the cross section of the neutron scattering. It is very helpful to the discussions in the following sections.

In the incoherent inelastic neutron scattering (INS), the cross section of the l-phonon process is expressed as follows [67]:

\[
\frac{d^2\sigma}{d\Omega dE}|_{inc, l-phonon} = \frac{\sigma_{inc} k^l}{4\pi} \frac{1}{k^2 m} e^{-\frac{Q^2}{2m}} \sum_s \frac{(\hat{q} \cdot \hat{\phi}_s)^2}{\omega_s} \left( \langle \eta_s \rangle + 1 \right) \delta(\omega - \omega_s), 
\]

(3.1)
where \( \sigma_{inc} \) is the incoherent scattering cross section, \( k \) and \( k' \) are the wavenumber of the incident and scattered neutrons, respectively. \( Q \) is the momentum transfer. The subscript "s" represents a specific normal mode \( s \), \( \hat{e}_s \) is the polarization of the mode \( s \), \( \omega_s \) is the frequency of the mode \( s \). Notice that the above equation only gives the phonon-emission term. To obtain the phonon-absorption term, one just needs to change the last two terms in Eq. 3.1 to \( \langle n_s \rangle \delta(\omega + \omega_s) \). \( \langle n_s \rangle \) is the occupation number in the state \( s \):

\[
\langle n_s \rangle = \frac{1}{e^{\hbar \omega_s/k_B T} - 1}.
\]

Here we introduce the density of state \( G(\omega) \). \( G(\omega) d\omega \) is the probability that one mode appears in the energy range of \( \omega \) to \( \omega + d\omega \). \( G(\omega) \) is normalized:

\[
\int_0^{\omega_M} G(\omega) d\omega = 1,
\]

where \( \omega_M \) is the cut-off frequency. According to the definition of \( G(\omega) \), one can write down another expression for \( G(\omega) \):

\[
G(\omega) = \frac{1}{3N} \sum_s \delta(\omega - \omega_s),
\]

where \( N \) is the number of the unit cell in the condensed matter. And the total number of modes is \( 3N \).

One can change the form of Eq. 3.1 with the definition of \( G(\omega) \):

\[
\frac{d^2\sigma}{d\Omega dE} \bigg|_{inc,1-phonon} = \sigma_{inc} k' \frac{1}{2m} e^{-\frac{Q^2 x^2}{2m}} 3N \int \frac{(\hat{Q} \cdot \hat{e}_s)^2}{\omega_s} [\langle n_s \rangle + 1] \delta(\omega - \omega_s) G(\omega_s) d\omega_s
\]

\[
= \sigma_{inc} k' \frac{1}{2m} e^{-\frac{Q^2 x^2}{2m}} 3N \frac{(\hat{Q} \cdot \hat{e}_s)^2}{\omega} [\langle n \rangle + 1] G(\omega),
\]

where \( \langle (\hat{Q} \cdot \hat{e}_s)^2 \rangle \) is just the average of \( (\hat{Q} \cdot \hat{e}_s)^2 \) with respect to all the modes with a frequency of \( \omega \). For systems with high symmetry or isotropic system, \( \langle (\hat{Q} \cdot \hat{e}_s)^2 \rangle \) is equal to \( Q^2/3 \) (This form is different for the system that has more than 1 atom in the unit cell). Therefore, the above equation can be simplified as:

\[
\frac{d^2\sigma}{d\Omega dE} \bigg|_{inc,1-phonon} = N \sigma_{inc} k' \frac{1}{2m} e^{-\frac{Q^2 x^2}{2m}} \frac{Q^2}{\omega} [\langle n \rangle + 1] G(\omega).
\]

It is well-known that the relation between the incoherent inelastic cross section and the self dynamic structure factor \( S_d(Q, \omega) \) is expressed as [67]:

\[
42
\[
\frac{d^2\sigma}{d\Omega dE|_{inc}} = N \frac{\sigma_{inc} k'}{4\pi} \frac{1}{k} S_{e}(Q, \omega) .
\] (3.7)

Combining the above two equations, one can obtain the following expression for \(G(\omega)\):

\[
G(\omega) = \frac{2m}{e(-Q^2\omega^2)} \frac{\omega S_{e}(Q, \omega)}{Q^2[(n)+1]} .
\] (3.8)

If \(Q\) is small enough, the Debye-Waller factor, \(e(-Q^2\omega^2)\), in above equation can be ignored, then we can approximate the above result as follows:

\[
G(\omega) \approx \frac{2m}{\hbar} \frac{\omega S_{e}(Q, \omega)}{Q^2[(n)+1]} .
\] (3.9)

In the classic limit \(k_B T \gg \hbar \omega\), the above expression can be further approximated as follows:

\[
G_{cl}(\omega) = \lim_{k_B T \to \hbar \omega} G(\omega) = \frac{2m}{k_B T} \lim_{Q \to 0} \frac{\omega^2}{Q^2} S_{e}(Q, \omega) .
\] (3.10)

One can introduce the so-called reduced vDoS:

\[
g(\omega) = \frac{G(\omega)}{\omega^2} .
\] (3.11)

It is easy to find that, in the classic limit and small \(Q\) limit, \(g(\omega)\) and \(S_{e}(Q, \omega)\) has a very simple relation:

\[
g_{cl}(\omega) = \frac{2m}{k_B T Q^2} S_{e}(Q, \omega) \propto S_{e}(Q, \omega) .
\] (3.12)

For the materials containing H atom, their INS spectra are dominated by the incoherent contribution due to the exceptionally large incoherent cross section of H atom. According to Eqs. 3.9 - 3.12, their INS spectra give direct information on the vDoS of the systems. In fact, vDoS plays an important role in the studies of various kinds of hydrogenous materials.

### 3.2 Boson Peak in One-Phase Region

Boson peak is a broad peak observed at frequencies \(\sim 2\) to \(10\) meV in the inelastic neutron [68-71], nuclear inelastic [72-75] and Raman [76-79] scattering spectra of disordered materials and supercooled liquids. Its origin is widely believed to be related to the transverse dynamics of the material [73-75, 80, 81]. Moreover, both theoretical and experimental studies assign the boson peak in glass to a phenomenon
reminiscent to the van Hove singularity of the transverse phonon of the crystal counterpart [74, 82, 83]. It is worth mention that, boson peak has dependence on the density of the material: as density increases, the frequency of the boson peak increases and the height decreases [69-72, 77, 84]. Then considering that the order parameter of the hypothetical LLT is just the density [12], the boson peak provides a good way to examine the existence of the liquid-liquid transition.

As the first step, we measure the boson peak in the one-phase region. In Section 1.3, I review the preliminary result of the boson peak measurement in the confined water. It is found that the measured INS spectra include a boson peak at about 6 meV, which only emerges below ~230 K (see Fig. 1.8) [33]. Interestingly, this temperature of emergence of boson peak (denoted as \( T_B \)) is very close to the Widom line temperature determined by the dynamic crossover, which is 225 K at ambient pressure [31]. A similar result was also observed in a computer simulation study [85]. However, the situation at high pressures is still unclear. In order to figure out the relation between the emergence of the boson peak and the Widom line, we performed a series of INS experiments at higher pressures from 400 to 2400 bar employing the Disk Chopper Spectrometer (DCS) [86] at the National Institute of Standards and Technology Center for Neutron Research [48]. The measured spectra were analyzed by a model that consists of an elastic component, a quasi-elastic component and an inelastic component. The quasi-elastic component is represented by the Relaxed Cage Model [87], and the inelastic component is represented by the Damped Harmonic Oscillator model [88].

The left set of panels in Fig. 3.1 shows measured INS spectra \( S_m(Q,E) \) of the confined water at \( Q = 2 \) Å\(^{-1}\) and corresponding fitted curves, under different temperatures and pressures. The right set of panels shows the theoretical incoherent dynamic structure factors extracted from the fit: \( S_m(Q,E) = n_Q S_Q(Q,E) + n_I S_I(Q,E) \) (\( n_Q \) and \( n_I = 1 - n_Q \) are the fractional quasi-elastic and inelastic contributions respectively). In order to study the relation between the emergence of the Boson peak and Widom line crossing in deeply-cooled confined water, we first define the emergence of the Boson peak as follows: if there is a maximum in the \( S_m(Q,E) \) curve between ~2 and 10 meV we state that the Boson peak is visible or that the Boson peak emerges [e.g. the 200 K case in Fig. 3.1 (b2)]; on the other hand, if the \( S_m(Q,E) \) curve does not have
a maximum, decreasing monotonically in this $E$ range, we state that the Boson peak is not visible [e.g. the 225 K case in Fig. 1 (b2)].

Figure 3.1. INS spectra of the confined water system at $Q = 2$ Å$^{-1}$ at different $T$ and $P$. For each row, the left panel shows the measured spectrum $S_m(Q,E)$ and corresponding fitted curves under a specific pressure; for clarity the data have been shifted vertically by a fixed interval between adjacent temperatures. The right panel shows the corresponding theoretical curves $S_{th}(Q,E)$ extracted from the fit. The curve whose temperature is closest to $T_B$ is indicated for each pressure in the right set of panels.

The above definition can be illustrated better if one considers the derivative of $S_{th}(Q,E)$ with respect to $E$, $\partial E S_{th}(Q,E)$. A Boson peak is observed if $\partial E S_{th}(Q,E)$ has two roots with $E > 0$ [e.g. the 200 K case in
Fig. 3.2 (a1)); similarly no peak is observed if \( \partial_{\beta} S_{ab}(Q,E) \) has no roots at \( E > 0 \) [e.g. the 225 K case in Fig. 3.2 (a1)]. The intermediate condition, that \( \partial_{\beta} S_{ab}(Q,E) \) has just one root with \( E > 0 \), or equivalently that the maximum value of \( \partial_{\beta} S_{ab}(Q,E) \) (max[\( \partial_{\beta} S_{ab}(Q,E) \)]) just equals to 0, corresponds to the critical case that the Boson peak just emerges. We denote the temperature at which the Boson peak just emerges as \( T_B \). In the right column of Fig. 3.1, we mark the \( S_{ab}(Q,E) \) curve whose temperature is closest to \( T_B \) for each pressure. One may estimate the value of \( T_B \) better with a linear interpolation method. Take 1200 bar case for example, from Fig. 3.2 (a2) it is found that the value of \( \text{max}[\partial_{\beta} S_{ab}(Q,E)] \) changes smoothly and monotonically as \( T \) changes, therefore one can perform a linear interpolation with respect to \( \text{max}[\partial_{\beta} S_{ab}(Q,E)] \) and \( T \) to find out the approximate value of \( T_B \) at which \( \text{max}[\partial_{\beta} S_{ab}(Q,E)] \) vanishes. With this method we obtain \( T_B = 219.8 \pm 1.3 \) K under 1200 bar, as shown in Fig. 3.2 (a2). Another example is provided by Fig. 3.2 (b2) where \( T_B \) is estimated to be \( 211.2 \pm 1.1 \) K under 2000 bar.

Figure 3.2. An illustration of the definition of \( T_B \). For each row, the left panel shows the derivatives of \( S_{ab}(Q,E) \) with respect of \( E, \partial_{\beta} S_{ab}(Q,E) \), under a specific pressure. Corresponding maxima of \( \partial_{\beta} S_{ab}(Q,E) \) at different temperatures under this pressure are denoted by black squares in the right panel. The linear interpolation method for estimating \( T_B \) is illustrated in the right panel.

The values of \( T_B \) obtained using the method just described are denoted by squares in Fig. 3.3. The fragile-to-strong dynamic crossover (FSC) temperatures \( T_z \) at different pressures are also shown (as
Below ~1600 bar the solid curve, which represents the behavior of $T_B(P)$, is nearly parallel to the blue dashed curve that represents the profile of $T_s(P)$. This suggests a strong correlation between the emergence of the Boson peak and the FSC. Note that, the computer simulation study shows that in water the locus of the FSC coincides with the Widom line [30]. Thus we conclude that below ~1600 bar, the emergence of the Boson peak is strongly correlated to the Widom line in the sense of the LLCP hypothesis, even though they do not overlap.

Figure 3.3. $T_B(P)$ in the $P$-$T$ plane. The squares represent interpolated estimates of $T_B(P)$. The triangles demarcate the FSC transition. The star denotes the estimated location of the LLCP, obtained from the FSC measurements [31]. The two dotted lines denote the extrapolations of the two points at $P=2000$ bar and 2400 bar and of the two points at $P=1200$ bar and 1500 bar.

From Fig. 3.3 it is evident that above ~1600 bar, the slope of the $T_B(P)$ profile is different from the slope below ~1600 bar: the positions of $T_B(P)$ under $P = 2000$ and 2400 bar deviate considerably from the extrapolation of the lower pressure cases. This deviation corresponds to the experimental result that above ~1600 bar the FSC disappears [31]. Moreover, this deviation suggests that the Widom line ends between 1500 bar and 2000 bar since if the Widom line continues to develop at pressures higher than 2000 bar, then due to the strong correlation between $T_B(P)$ and the Widom line, the profile of $T_B(P)$ will smoothly develop at higher pressures in a fashion similar to that of the Widom line. In the LLCP hypothesis, the
Widom line ends at the LLCP, which produces a singularity. In this sense, the profile of $T_b(P)$ is not necessarily smooth at the critical pressure. Thus one may estimate the critical pressure by finding the intersection point of the extrapolations of the two points at $P = 2000$ bar and 2400 bar and of the two points at $P = 1200$ bar and 1500 bar. As shown in Fig. 3.3, the critical pressure estimated in this way is 1592 bar, which is consistent with the result of 1600±400 bar estimated by detecting the end-pressure of the FSC [31].

In summary, in the one-phase region of the phase diagram, the emergence of the boson peak tracks the Widom line of the possible liquid-liquid transition determined by FSC below the critical pressure. And the locus of the emergence of the boson peak in the $P$-$T$ plane changes the slope at the critical pressure. These observations strongly suggest that the behaviors of boson peak may response to the existence of the liquid-liquid transition in the deeply cooled confined water.

3.3 Boson Peak in Two-Phase Region

Encouraged by the results of the boson peak measurement in the one-phase region of the phase diagram, we performed a series of experiments on measuring the boson peak of the confined water in the two-phase region of the phase diagram [89]. The experiments were performed at the Cold Neutron Chopper Spectrometer (CNCS) [90] at Spallation Neutron Source, Oak Ridge National Laboratory. Figure 3.4 (a) shows the phase diagram of the confined heavy water [47]. The measured points at the two-phase region are denoted in Fig. 3.4 (a). Notice that, in the boson peak study, we measure the confined H$_2$O rather than D$_2$O. However, here we still perform the boson peak measurement based on the phase diagram of the confined D$_2$O for the following reasons: (1) we cannot obtain the phase diagram of the confined H$_2$O with the method used in Ref. [47] owing to the exceptionally large incoherent cross section of H atom. (2) The difference between the phase diagrams of confined H$_2$O and D$_2$O is expected to be several kelvins due to the isotope effect [91]. In fact, the conjectured phase diagram of deeply cooled bulk D$_2$O is quite similar to that of the bulk H$_2$O [92].
Figure 3.4. (a) Phase diagram of the deeply cooled heavy water confined in MCM-41 [47]. The inset shows the two-dimensional hexagonal structure of MCM-41. The red circles denote the measured points in the LDL phase. These points are approached by first cooling the system to desired temperature and then pressurizing (red arrow). The blue triangles denote the measured points in the HDL phase. These points are approached by cooling the system to desired temperature at desired pressure (blue arrow). With these approaches we avoid crossing the phase boundary directly. (b) The measured INS spectra at T = 165 K, and P = 2, 3, 4, and 4.7 kbar. (c) Model fitting of the measured $S(Q,E)$ with Eq. 3.13 at $Q = 2 \text{ Å}^{-1}$, $T = 165 \text{ K}$, $P = 2 \text{ kbar}$.

Figure 3.4 (b) shows the measured boson peak at 165 K and under 2, 3, 4 and 4.7 kbar. According to the phase diagram in Fig. 3.4 (a), the former two points are in the LDL phase, while the latter two are in the HDL phase. It is seen that, the height of the boson peak decreases as pressure increases in the energy range from 2 to 9 meV. This pressure dependence reverses at higher energies. Moreover, the largest difference between the spectra of adjacent pressures is found between 3 and 4 kbar. In order to quantitatively analyze the data, we use the following equation to fit the measured INS spectrum at a specific Q:
\[ S_{s,m}(Q, E) = R(E) \otimes \left\{ \frac{1}{\pi} \frac{A_1 Y_1}{E^2 + Y_1^2} + \frac{A_2}{\sqrt{2\pi}\sigma_{BP}E} \cdot \exp \left( -\frac{(E_{BP}-\ln E)^2}{2\sigma_{BP}^2} \right) \right\} \cdot D(E), \tag{3.13} \]

where \( S_{s,m}(Q, E) \) is the measured self dynamic structure factor of the confined water, \( R(E) \) is the energy resolution function. In the square brackets, the first term is a Lorentzian function, which represents the quasi-elastic contribution; the second term is a log-normal distribution function. \( \sigma_{BP} \) and \( E_{BP} \) relate to width and position, respectively. \( A_1 \) and \( A_2 \) are the amplitudes of these two parts. \( D(E) \) is the detailed balance factor and is expressed as \( \exp(E/2k_BT) \) \[67\]. Log-normal distribution function can describe the boson peak well \[93\] and has been applied to the confined water successfully \[68\]. A typical fit is shown in Fig. 3.4 (c).

Since a probability distribution function is used to represent the boson peak, it is convenient to define the mean frequency \( M \) and the variance \( V \) of the boson peak as follows \[94\]:

\[
M = \exp \left( E_{BP} + \frac{\sigma_{BP}^2}{2} \right) \tag{3.14}
\]

\[
V = \left[ \exp(\sigma_{BP}^2) - 1 \right] \cdot \exp(2E_{BP} + \sigma_{BP}^2). \tag{3.15}
\]

\( M \) and \( V \) denote the position and the broadness of the boson peak in the frequency domain, respectively. In addition, we define the maximum of the log-normal distribution function in Eq. 3.13 as the height of the boson peak \( (H) \).

Figure 3.5 (a), (b) and (c) show the values of \( M, H, \) and \( V \) of the boson peaks at the measured points shown in Fig. 3.4 (a) respectively. For both measured temperatures, as pressure increases, \( M \) and \( V \) increase, while \( H \) decreases. These dependences on pressure, or density, agree to the observations in other experimental \[69-72, 77, 84\] and computer simulation studies \[80, 81\]. Particularly, as the pressure changes from 3 to 4 kbar, all these quantities undergo larger changes. This phenomenon strongly suggests an abrupt increase in density as the pressure changes from 3 to 4 kbar, and is consistent with the observation that there is a LDL to HDL transition from 3 to 4 kbar at \(-170 \text{ K}\) in confined water \[47\]. For comparison, we also show the average density of the confined D\(_2\)O as a function of pressure at 170 K (obtained by the same method used in ref. [47]).
Figure 3.5. (a) Mean frequencies \( M \), (b) heights \( H \) and (c) variances \( V \) of the boson peaks at the measured points shown in Fig. 3.4 (a). (d) The average density of the confined D\(_2\)O at \( T = 170 \) K and \( P = 2, 3, 4 \) and 5 kbar.

The line shapes of the boson peak extracted from the fit \( S_{\text{bos}}(Q,E) \) at \( T = 165 \) K, \( Q = 2 \) Å\(^{-1} \) and \( P = 2, 3, 4, \) and 4.7 kbar are shown in Fig. 3.6 (a). The red curve in Fig. 3.6 (a) shows the difference between the boson peaks at 4.7 (HDL) and 2 kbar (LDL). In addition, we show the reduced vDoS \( g(E) \) of LDA and HDA measured by INS and their difference in Fig. 3.6 (b) [95]. According to Eq. 3.12, in the classic limit, the self dynamic structure factor \( S_s(Q,E) \) is related to the reduced vDoS \( g(E) \) as follows:

\[
g(E) \propto \lim_{Q \to 0} \frac{1}{Q^2} S_s(Q,E). \tag{3.16}
\]

Thus we can compare \( g(E) \) with \( S_s(Q,E) \). Note that, according to Eq. 3.8, the effect of Debye-Waller factor should be considered. However, the mean square displacements \(< x^2 >\) of HDA and LDA are found to be very small [96], thus the Debye-Waller factors contained in \( g(E) \) of LDA and HDA can be ignored. It is significant that, the spectral difference of the confined water shown in Fig. 3.6 (a) resembles the one of the amorphous ices shown in Fig. 3.6 (b). This similarity is consistent with the hypothesis that the LDL
and HDL are thermodynamic extensions of LDA and HDA to liquid state [4]. The amplitude of the difference of the confined water is smaller than that of the amorphous ices. This is partially because (1) only the free water part in the confined water undergoes a liquid-liquid transition [47]; and (2) the confinement can suppress the phase transition [42]. Generally, the INS spectrum reflects the strength of the hydrogen bonds between water molecules. Therefore, the similarity of the spectral differences between the HDL-LDL case and HDA-LDA case suggests that the difference of local structure between LDL and HDL is similar to that between LDA and HDA. In fact, Soper and Ricci [29] show that the principal difference between the local structures of LDL and HDL is that, the first peak in the $g_{OO}(r)$ structure factor is barely altered in position, while the second peak position becomes smaller from LDL to HDL. And a similar change in $g_{OO}(r)$ from LDA to HDA is also found [97].

A theoretical study [98] shows that the boson peaks at different temperatures and pressures can be rescaled to one master curve with a characteristic energy $E_c$ by the following way:

![Figure 3.6.](image)

(a) Line shapes of the boson peak extracted from the fit ($S_{BW}(Q,E)$) at $T = 165$ K, $Q = 2$ Å$^{-1}$ and at $P = 2$ (brown), 3 (blue), 4 (green), and 4.7 (pink) kbar. The red curve shows the spectral difference between 4.7 and 2 kbar. (b) The reduced vDoS of HDA (green circles) and LDA (blue squares) [95] and their difference (red triangle). The amplitudes are rescaled for comparing with the boson peaks of the confined water.
\[ E \rightarrow \varepsilon = \frac{E}{E_c}, g(E) \rightarrow g(\varepsilon) = E_c^2 g(E(\varepsilon)). \]  

(3.17)

We perform a similar rescaling on \( S_{Br}(Q,E) \) of the confined water shown in Fig. 3.6 (a). The fitting parameter \( E_{Br} \) in Eq. 3.13 is employed as the characteristic energy \( E_c \). The result is shown in Fig. 3.7. It can be seen that, even the values of \( H, M \) and \( V \) are different, the curves obtained in LDL region can be rescaled to one master curve, and the curves in HDL region can be rescaled to a different one. A common master curve for all measured boson peaks in LDL (or HDL) suggests a common mode distribution, and reflects the similarity in dynamic behavior between different measured points. The failure in rescaling the boson peaks to one master curve is attributed to the difference in the local structure [70, 72]. Thus, the existence of two different master curves supports the existence of two structurally different liquid phases in the confined water.

In summary, we measure the boson peak of confined water at low temperatures and high pressures to examine the existence of the liquid-liquid transition in the confined water. The behaviors of boson peak
suggest a transition from LDL to HDL between 3 and 4 kbar at ~170 K. This result is consistent with the phase diagram found in the confined heavy water by density measurement [47].

3.4 Librational Band in Vibrational Density of State

We have also studied the librational band in the vibrational density of state of the confined water at the two-phase region with INS. The measured points are at $T = 170$ K and at $P = 2, 3, 4, \text{ and } 4.8 \text{ kbar}$. The experiment was performed at the vibrational spectrometer (VISION) at Spallation Neutron Source, Oak Ridge National Laboratory [99]. VISION has a very good performance from the low-frequency region to about 200 meV. The spectra of the confined water measured at VISION are shown in Fig. 3.8.

![Figure 3.8. vDoS of the confined water measured at VISION. The measured temperature is 170 K. The black, red, blue and green curves represent the spectra at $P = 2, 3, 4, \text{ and } 4.8 \text{ kbar}$, respectively.](image)

In Fig. 3.8, the peak located between 2 to 15 meV is just the boson peak. From Fig. 3.8 one can find that the pressure dependence of the boson peak obtained at VISION is very similar to that obtained at CNCS. The low-frequency region of the vDoS is also called translational band because it mainly reflects the collective translational motion of the condensed matter. The librational band is in the energy range
from about 50 to 120 meV. This band represents the “hindered rotation” of the water molecule [100]. It is faster and more local than the motions reflected in the translational band.

Figure 3.9. (a1) Librational bands of confined water at $T = 170$ K and $P = 2, 3, 4,$ and $4.8$ kbar. (a2) The spectral differences between the adjacent pressures. (b1) Librational bands of LDA and HDA. (b2) The spectral difference between HDA and LDA. The data of amorphous ices are rescaled for comparing with the data of the confined water.

Here I emphasize the librational band separately in Fig. 3.9 (a1). The spectral differences between adjacent measured pressures are shown in Fig. 3.9 (a2). It can be seen that the energy of the low-energy side of the librational band measured at 4 kbar (HDL) is lower than that measured at 3 kbar (LDL) by a few meV. The librational band of the confined water has also been measured at ambient pressure [101, 102]: as crossing $-225$ K from low temperature side, the change in librational band is very similar to the spectral change from 3 kbar (LDL) to 4 kbar (HDL) shown in Fig. 3.9 (a2). Mallamace et al. [28] show that as crossing 225 K from low temperature side, the local structure of water transforms from a predominately LDL form to a predominately HDL form. Therefore, our result is consistent with the
previous measurements and suggests the existence of the LDL and HDL. The spectral difference observed here suggests that the hydrogen bond between the central water molecule and first shell becomes weaker from LDL to HDL. Previous studies on aqueous organic solutions [40, 41] show that the OH-stretching mode is softer in LDL than that in HDL. This phenomenon suggests that the hydrogen bond between the central water molecule and the first shell becomes weaker from LDL to HDL. Our result is thus consistent with the result in the aqueous organic solutions.

We show the vDoS in librational band of LDA and HDA in Fig. 3.9 (b1) and their spectral difference in Fig. 3.9 (b2) [103-105]. The low-energy side of the librational band of confined water is much smoother than those of the amorphous ices and the ice Ih (the spectrum of ice Ih, which is very similar to that of the LDA, is not shown here). This phenomenon suggests that the confined H$_2$O is in liquid phase. The spectral change from LDA to HDA is similar to that from LDL to HDL (but with much larger amplitude): there are excess modes between $\sim$45 and $\sim$65 meV in the vDoS of HDA comparing with that of LDA because of the red shift of the low-energy side of the librational band from LDA to HDA. This spectral change is assigned to larger O-O distance [97] and weaker hydrogen bond from LDA to HDA [104].

3.5 Slow Dynamics

Last three sections focus on the fast motions of the confined water, i.e., the motions of water molecules with sub-picosecond time scale. In this section I will discuss the slow dynamics of the confined water and its possible relation to the hypothetical liquid-liquid transition.

As introduced in Section 1.3, Prof. Chen’s group studied the slow dynamics of the confined water with QENS technique [24, 31]. They found that in the pressure range from 1 bar to 1.6 kbar, the characteristic relaxation time of the confined water undergoes a dynamic crossover from the super-Arrhenius behavior at high temperatures to the Arrhenius behavior at low temperatures. They attributed the dynamic crossover to the change of the local structure of the confined water and assigned the
crossover temperature to the locus of the Widom line [30, 31]. The dynamic crossover disappears when
the pressure is higher than 1.6 kbar. The disappearance of the dynamic crossover is ascribed to the
beginning of the phase separation. Therefore, the critical pressure should be at about 1.6 kbar.

The previous experimental studies [31, 102] on the slow dynamics of the confined water are up to
2.4 kbar. It is interesting to know the situation at higher pressures. From the phase diagram shown in Fig.
2.5 one can find that, at pressures higher than 3.5 kbar, the confined water is dominated by the HDL
phase. According to Ref. [54], HDL and LDL phases exhibit different glassy behaviors at low
temperatures. The study of the relaxation behavior with QENS can bring direct information on the glassy
behavior of the HDL phase.

We perform a QENS experiment at the High-Flux Backscattering Spectrometer (HFBS) at National
Institute of Standards and Technology Center for Neutron Research on the confined water at 4 kbar. We
also measure the confined water system at 1 bar for comparison.

The normalized scattering spectra of the confined water, \( I_{H_2O}(E) \), were fit with the following
equation:

\[
I_{H_2O}(E) = \left\{ p\delta(E) + (1-p)FT[F_s(t)] \right\} \otimes R(E). \tag{3.18}
\]

In the above equation, \( FT \) denotes a time Fourier transform, \( R(E) \) is the energy resolution function,
\( \otimes \) is the convolution operator, and \( p \) is the elastic fraction. The existence of the elastic component is due
to the confinement [106]. \( F_s(t) \) is the self-intermediate scattering function (SISF). For supercooled water,
the translational contribution to \( F_s(t) \) can be modeled by stretched exponential decay form [87, 107, 108]:

\[
F_s(t) \approx \exp\left[-\left(t/\tau\right)^\beta\right], \tag{3.19}
\]

where \( \tau \) is the characteristic relaxation time of the long-time relaxation, and \( \beta \) is the stretching exponent.
Notice that the above approximation only works when \( Q \) is smaller than 1 \( \text{Å}^{-1} \) at where the local motions
can be ignored [31, 87]. The mean characteristic relaxation time \( \langle \tau \rangle \) is calculated from \( \tau \) and \( \beta \) by \( \langle \tau \rangle = \beta^{-1} \Gamma(\beta^{-1}) \tau \). In this study, \( \beta \) is fixed to 0.5, just as in the analyses of Bertrand et al. [107] and Yoshida et
The value of $p$ was obtained from the fit of the spectrum at the highest measured temperature, since the elastic fraction $p$ is most easily distinguished at the highest temperature.

Figure 3.10. Measured QENS spectra of the confined water system along with the corresponding calculated curves fitted by Eq. 3.18 at $Q = 0.75 \, \text{Å}^{-1}$. Panels (a) and (b) show the measured spectra and the calculated curves in logarithmic scale for $P = 1$ bar and 4 kbar, respectively. Panel (c) shows the measured spectra and the calculated curves in linear scale for four specific conditions.

Figure 3.10 shows the measured spectra of the confined water system along with the corresponding calculated curves fitted by Eq. 3.18 at $Q = 0.75 \, \text{Å}^{-1}$. Figure 3.11 (a) and (b) show the Arrhenius plots of
(τ) at $Q = 0.75 \text{ Å}^{-1}$ for $P = 1$ bar and 4 kbar, respectively. In Fig. 3.11 (a), the higher-temperature data exhibit super-Arrhenius behavior, which can be fitted by the Vogel-Fulcher-Tammann (VFT) relation, $(\tau) = \tau_0 \exp[DT_0/(T - T_0)]$. The lower-temperature data exhibit Arrhenius behavior, which can be fitted by the Arrhenius relation, $(\tau) = \tau_0 \exp(E_a/k_BT)$. In this case, the crossing temperature of these two fits, i.e. the dynamic crossover temperature $T_c$, is 225 K, which is very close to the value obtained in previous studies, 224 K [31, 102]. At 4 kbar, we also find a dynamic crossover, which takes place at 219 K. Note that, there is no liquid-liquid transition or Widom line at such high pressure according to the phase diagram in Fig. 2.5. Nevertheless, this is not very unexpected, because the dynamic crossover does not necessarily relate to a Widom line or a phase transition.

![Figure 3.11. Arrhenius plot of the mean characteristic relaxation time (τ) at (a) 1 bar (red circles) and (b) 4 kbar (black squares) at $Q = 0.75 \text{ Å}^{-1}$. The dynamic crossover takes place at about 225 K in the former case and at about 219 K in the latter case.](image-url)
From Fig. 3.11 one can find that the $T$ dependence of $\langle \tau \rangle$ at 1 bar exhibits a more evident super-Arrhenius feature than that at 4 kbar. This point can be seen in the measured spectra. From Fig. 3.10 (c) it is found that, at 266 K, the height of the measured spectrum at 1 bar is 0.02367, which is slightly smaller than that at 4 kbar, 0.02475. It indicates that at 266 K the relaxation at 1 bar is a little faster than that at 4 kbar. However, this ordering reverses at lower temperatures. At 220 K, the height of the measured spectra at 1 bar is 0.09044, which is considerably larger than the one at 4 kbar, 0.07572. It shows that at 220 K the relaxation at 1 bar is significantly slower than that at 4 kbar. The super-Arrhenius behavior of the confined water is related to the collective relaxation process of water [107]. A water molecule is likely to be trapped in a cage consisting of its neighboring molecules. The translational diffusion of this molecule requires cage breaking that is associated with simultaneous breaking of several hydrogen bonds. As $T$ decreases, the local order surrounding a molecule is enhanced, which leads to a significant increase of the structural relaxation time [87]. Subsequently, this collective relaxation process exhibits a non-Arrhenius $T$ dependence. For the case of $P = 1$ bar, as $T$ decreases, the local structure of water gradually transforms from normal form to predominately LDL form [47]. The water molecule in the LDL phase has an open tetrahedral hydrogen-bond network up to its second coordination shell [29]. Such a strong local order will weaken the fluidity of water molecule and lead to an evident super-Arrhenius behavior. On the contrary, at 4 kbar, as $T$ decreases, the local structure of water transforms to a HDL form [47]. A water molecule in the HDL phase forms a tetrahedral hydrogen-bond network with its first coordination shell, while the second coordination shell collapses [29]. In this case, the local order is relatively weak, which lead to a weak super-Arrhenius behavior.

At temperatures lower than $T_s$, the activation energy $E_a$ at 1 bar is 16.5 kcal/mol, which is larger than the value obtained at 4 kbar, 13.9 kcal/mol. This phenomenon suggests that the strength of the hydrogen bond in LDL is stronger than that in HDL. In the last section we present the librational band in the vibrational density of state of the confined water. It shows that the energy of the low-energy side of the librational band measured in the HDL is lower than that measured in LDL by a few meV. This spectral difference indicates that the hydrogen bond becomes weaker from LDL to HDL. Therefore, the Arrhenius
behaviors of the \( \langle \tau \rangle \) found in this study are consistent with the result of the measurement on the librational band.

Figure 3.12 shows the positions of the dynamic crossovers in the \( P-T \) plane. As mentioned before, the dynamic crossover disappears at pressures higher than 1.6 kbar \([31, 102]\). In this work, we show that the dynamic crossover reappears at 4 kbar. In addition, the position of the dynamic crossover at 4 kbar deviates from the extrapolation of the positions of \( T_c(P) \) below 1.6 kbar significantly. Both of these two phenomena indicate a discontinuity between 1.5 and 4 kbar. According to Fig. 2.5, there is a liquid-liquid transition between 1.3 and 3.5 kbar. For this reason, it is reasonable to ascribe the abnormal behaviors of \( T_c(P) \) to the existence of the phase separation in the confined water.

Figure 3.12. \( T_c(P) \) in the \( P-T \) plane. The black squares represent the positions of the dynamic crossover observed by Liu et al. \([31, 102]\). The red circles represent the positions of the dynamic crossover observed in this study. Notice that, according to Liu et al. \([31, 102]\), the dynamic crossover disappears from 1.6 kbar to the highest pressure they measured, which is 2.4 kbar.

In summary, in this section we investigate the single-particle dynamics of the water confined in MCM-41 at 4 kbar with QENS. A dynamic crossover phenomenon is observed at 219 K. By comparing this dynamic crossover with the one observed at ambient pressure we find that: (a) above the crossover temperature, the temperature dependence of the characteristic relaxation time at ambient pressure exhibits a more significant super-Arrhenius behavior than that at 4 kbar. (b) Below the crossover temperature, the
Arrhenius behavior found at ambient pressure has a larger activation energy compared to the one found at 4 kbar. We ascribe the former to the different local orders between LDL and HDL, and the latter to the different hydrogen-bond strengths between LDL and HDL. In addition, we find that the pressure dependence of the crossover temperature suggests a discontinuity between 1.5 and 4 kbar, which corresponds to the likely liquid-liquid transition observed between 1.3 and 3.5 kbar in confined D$_2$O with elastic neutron scattering. For these considerations, we conclude that the phenomena observed in this section are consistent with the liquid-liquid transition hypothesis.

3.6 Conclusion

In this chapter, using different neutron scattering techniques, we investigate various kinds of dynamic properties of the confined water. The time scale range of these motions is from sub-picosecond to hundreds of nanosecond, which is more than seven orders. The boson peak mainly reflects the translational collective motion of the water molecules. The frequency and shape of the boson peak are related to the density and the local structure of the disordered material. The motions corresponding to the librational band in the vibrational density of state are faster than the boson peak-related motions by one order. It represents the hindered rotational motions of water molecule. Its frequency depends on the strength of the hydrogen bond between the central molecule and the first shell, which is related to the very local structure of the system. The relaxation process has a time scale from tens of picosecond at room temperature to more than 1 microsecond at temperatures lower than 200 K. It has relations with the density and structure of the system. Our findings in this chapter are consistent with the phase diagram found in Chapter 2. More importantly, these dynamic properties provide information on the configuration and strength of the hydrogen bond in the confined water, which cannot be obtained from density measurement.
Chapter 4  Dynamics of Protein-Water System

"...everything that living things do can be understood in terms of the jigglings and wigglings of atoms."

—R. P. Feynman

4.1 Introduction

After the discovery of the molecular structure of DNA using X-ray diffraction in 1953 [109], the crystallographic structures of many kinds of biological macromolecules, such as DNA and proteins, have been investigated extensively. These studies confirm the crucial role that structure plays in the establishment of the functions of biological macromolecules. Later on, the importance of dynamics in biological activity started to be appreciated [110]. Intuitively, biological macromolecules cannot be totally static, and a "rigid body" cannot have any biological activity. Now it is well accepted that both the structure and dynamics are essential to the enzymatic functions of proteins [111]. Protein dynamics can be considered as any time-dependent change in the protein atomic coordinates. By introducing the concepts of conformational substate and energy landscape, Frauenfelder et al. [112-115] proposed a picture for the study of protein dynamics. In this framework, the energy landscape describes the potential energy of a protein system as a function of conformational coordinates, i.e., a hyper-surface in the high-dimensional space of the coordinates of all atoms in a protein system. A protein system with a particular energy landscape can assume a very large number of nearly isoenergetic conformational substates. The probabilities of conformational substates and the energy barriers between them are determined by the energy landscape. Due to the complexity of the protein system, the shape of the energy landscape is extremely complicated and can be divided into several tiers. With these ideas, it is natural to divide protein dynamics into two main groups according to the time scale or, equivalently, to the tier of the energy landscape sampled [116]. (1) “Slow” dynamics (>1 µs) is characterized by fluctuations between kinetically distinct states that are separated by energy barriers of $k_BT$, i.e., large-amplitude collective
motions. Biological processes like enzyme catalysis and signal transduction occur on this time scale. (2) “Fast” dynamics (<1 μs, especially on the time scale of <1 ns) is characterized by fluctuations between conformational substates which are structurally similar and are separated by energy barriers much smaller than $k_B T$. Protein motions on this time scale are usually more localized and faster.

Compared with the “slow” functional motions of proteins, the motions corresponding to “fast” dynamics are usually of much smaller amplitude and significantly larger frequency. However, they are somehow connected to the “slow” motions that directly correspond to the enzymatic functions of proteins (the so-called “traffic model for conformational space” proposed by Frauenfelder et al. [117] provides some ideas about this connection). In the last few decades, protein dynamics on the “fast” time scale from 1 ps to 1 μs and its relation to biological activities were studied extensively by neutron scattering [118-120], nuclear magnetic resonance [121] and Mössbauer spectroscopy [122, 123]. The results show a strong correlation (but not sufficient and necessary conditions [124-126]) between the biological functions of proteins and atomic self-motion of constituent atoms.

Besides the hierarchy of the energy landscape, another striking feature of the protein dynamics is the importance of water [127]. For most proteins, they cannot perform their functions if they are not covered by a minimum amount of hydration water. Experimental studies show that the protein hydration water plays a key role in many aspects of protein dynamics, which include the mean square displacement of the protein constituent atom [128, 129], the subpicosecond collective vibrations [130-132], and the intraprotein $\alpha$ and $\beta$ relaxations [123, 133]. Thus the study of the protein hydration water is crucial for the understanding of protein dynamics.

In this chapter, I will present the studies on two kinds of motions in protein-water system. The first one is the intraprotein collective vibrational motions. In this part, I will discuss the relation between such motion and protein biological function, its hydration dependence and structural dependence. The result shows that hydration water is the key that governs the behavior of the intraprotein collective vibration. The second one is the slow dynamics of the protein hydration water. By investigating the hydration and temperature dependences of the characteristic relaxation process in protein hydration water, I confirm the
existence of the dynamic crossover in protein hydration water. Moreover, the relation between dynamic crossover in protein hydration water and the so-called “protein dynamic transition” [118] is revealed.

4.2 Intraprotein Longitudinal Phonon-Like Excitations

Protein exhibits phonon-like excitations based on the intraprotein collective vibrations [134]. These excitations can be detected by high-resolution inelastic X-ray scattering (IXS) technique due to the highly coherent signal of scattered X-ray photons [135]. A typical IXS spectrum of hydrated protein is shown in Fig. 4.1.

![IXS spectrum](image)

**Figure 4.1.** IXS spectrum of the hydrated lysozyme at $T = 300$ K, $h = 0.3$, and $Q = 6.09$ nm$^{-1}$. The red dots denote the measured data. The black curve denotes the energy resolution function. One can find that at around 10 meV there is a broad bump. This is the intraprotein longitudinal phonon-like excitation.

In order to extract the energy and damping rate of the phonon-like excitation, we use the Generalized Three Effective Eigenmode (GTEE) theory [136] to analyze the measured IXS spectra. In this theory, the normalized dynamic structure factor (NDSF) can be written as:
\[
\frac{S_{\text{GTEE}}(Q,E)}{S(Q)} = \text{Re} \left[ \frac{S_{\text{GTEE}}(Q,z)}{S(Q)} \right]_{z=iE} = \frac{1}{S(Q)} \text{Re} \left[ z + \frac{f_{un}(Q)}{z + z_u(Q) + \Sigma_{T}(Q)} \right]_{z=iE}^{-1}
\]

(4.1)

where \( f_{un}(Q) = Q v_0(Q) [S(Q)]^{1/2} \), \( v_0(Q) \) is the generalized thermal velocity, and \( S(Q) \) is the static structure factor. The subscripts \( u, n, \) and \( T \) denote momentum mode, number density mode and temperature mode, respectively. \( f_{un} \) and \( f_{uT} \) are the coupling constants between the \( u \) and \( n \) modes and the \( u \) and \( T \) modes, while \( z_u \) and \( z_T \) are decay rates of the \( u \) and \( T \) modes. The continued fraction form of the NDSF, Eq. 4.1, can be transformed into the three generalized hydrodynamic modes expression given by the following equation:

\[
\frac{S_{\text{GTEE}}(Q,E)}{S(Q)} = \frac{1}{\pi} \left[ A_h \frac{\Gamma_h}{\Gamma_h + E^2} + A_s \frac{\Gamma_s + b(E + \Omega_s)}{\Gamma_s + b(E + \Omega_s)^2} + A_{\Omega} \frac{\Gamma_{\Omega} - b(E - \Omega_s)}{\Gamma_{\Omega} + b(E - \Omega_s)^2} \right]
\]

(4.2)

In Eq. 4.2, the term proportional to \( A_h \) represents quasi-elastic scattering (Rayleigh peak) and the last two terms represent the inelastic scattering (Brillouin peaks). The fractional area of the Rayleigh peak is \( A_h = 1 - 2A_s \), where \( A_s \) is the fractional area of the Brillouin peak. The widths of the Rayleigh peak and the Brillouin peaks are given by \( \Gamma_h \) and \( \Gamma_s \), respectively, while \( \Omega_s \) is the position of Brillouin peak, i.e. the energy of phonon-like excitation (or equivalently the vibrational frequency). All the 6 parameters in Eq. 4.2 are functions of the 4 \( Q \)-dependent parameters: \( f_{un}(Q), z_u(Q), z_T(Q) \) and \( f_{uT}(Q) \), found in Eq. 4.1.

GTEE theory is a generalization of the three effective eigenmode theory [137] and has been successfully applied to study different biomaterials such as hydrated lipid bilayers [138] and liquid crystalline DNA [139]. The theoretical NDSF from GTEE, multiplied by detailed balance factor [\( \exp(E/2k_BT) \)] [67], then convoluted with energy resolution function, can be used to fit the measured IXS spectra. Figure 4.2 shows several examples of the fitting results for the hydrated lysozyme sample. The IXS data were obtained at 3-ID-XSD beamline [140-142] at the Advanced Photon Source, Argonne National Laboratory. The energy window is from -25 to 25 meV. The energy resolution of the instrument is about 1.5 meV. The experimental results based on the GTEE analysis are presented in the following paragraphs.
Figure 4.2. IXS spectra in logarithmic scale at three different $Q$ values ($Q = 6.79$, 24.92, and 29.89 nm\(^{-1}\) from left to right) and at the three hydration levels ($h = 0.06$, 0.33 and 0.43, from top to bottom). The blue points are the measured spectral data points. The pink line is the extracted NDSF (multiplied by the detailed balance factor). The green line shows the energy resolution function of the spectrometer. The cyan line is the fitted spectral intensity. The red arrows indicate the positions of the Brillouin peaks.

The bump at round 10 meV shown in Fig. 4.1 has a linear dispersion relation at $Q < 7$ nm\(^{-1}\) [143], which is similar to the longitudinal phonon-like excitation in liquid water [135]. A typical dispersion relation of such excitation is shown in Fig. 4.3.

The enzymatic function of protein depends on the temperature and hydration level [127]. For lysozyme, it only exhibits biological function when hydration level is larger than 0.2 and the temperature is higher than about 220 K. In order to clarify the role that the intraprotein collective vibrations play in the
establishment of the protein biological function, we measured the IXS spectra of lysozyme samples at different hydration levels and temperatures [132]. Figure 4.4 (a) shows the longitudinal phonon dispersion relations for lysozyme samples with four different hydration levels ($h = 0.06, 0.16, 0.33$ and $0.43$) at room temperature. Note that the first two hydration levels are lower than the minimum requirement for lysozyme to exhibit biological function ($\sim 0.2$), while the latter two are higher than the requirement. One can find that at $Q > -15$ nm$^{-1}$, the phonon exhibits a substantial softening for the higher hydration levels indicated by the lower energy. Meanwhile, the fractional area of the Brillouin peaks $A_s$ is significantly enhanced, as shown in Fig. 4.4 (b). Note that $A_s$ is related to the phonon population $n_p$ by $n_p(Q) \propto A_s(Q) \cdot S(Q)$, thus the increase of $A_s$ directly indicates an increase of phonon population.

Figure 4.3. Phonon dispersion relation of a lysozyme at $T = 300$ K and $h = 0.3$.

Figure 4.5 (a) shows the longitudinal phonon dispersion relations for lysozyme sample with $h = 0.3$ and at three different temperatures: 190 K, 240 K, and 300 K. Note that the first temperature is lower than the minimum temperature for lysozyme to exhibit biological function ($\sim 220$ K) while the latter two are higher than it. One can find similar phenomena as shown in Fig. 4.4. At $Q > -12$ nm$^{-1}$, the phonon energy exhibits a substantial softening as the temperature crosses the lowest temperature for biological function ($\sim 220$ K) from below. Meanwhile, the phonon population is enhanced.
Figure 4.4. (a) Measured dispersion relations of phonon-like excitations, and (b) fractional area of the Brillouin peaks $A_s$ at the four measured hydration levels at $T = 300$ K. Green squares, cyan rhombuses, red circles, and blue triangles indicate $h = 0.06, 0.16, 0.33$, and $0.43$, respectively. Within the $Q$ range of 20 - 31 nm$^{-1}$, the phonon energies are softened significantly and the phonon population is enhanced when crossing the minimum hydration level ($\sim 0.2$) required for the enzymatic function of lysozyme. However, within the $Q$ range of 3.5 - 13.5 nm$^{-1}$, such phenomena were not observed. The dashed lines are drawn to guide the eyes.

In summary, We have identified a well-defined softening of the phonon energies and an enhancement of the phonon population within a particular $Q$ range when crossing the threshold hydration level $h = 0.2 - 0.25$ from below at $T = 300$ K. Similar phonon energy softening and population enhancement also exist when crossing the minimum temperature for biological function from below at $h = 0.3$. These phenomena indicate that, for the lysozyme samples with native three-dimensional molecular structure, there may exist a correlation between the intraprotein short-time collective vibrational motions and the establishment of the enzymatic function of lysozyme. Collective modes observed in the $Q$ range of about 15 - 30 nm$^{-1}$ seem to be most sensitive to the establishment of enzymatic function of lysozyme.
Figure 4.5. (a) Measured dispersion relations of phonon-like excitations, and (b) fractional area of the Brillouin peaks $A$, at the three temperatures with $h = 0.3$. Red circles, blue rhombuses, and green triangles indicate $T = 300$, 240, and 190 K, respectively. When crossing the minimum temperature for biological function (~220 K) from below, the phonon energies are softened within the $Q$ range of about 13.5 - 31 nm$^{-1}$, and the phonon population is enhanced in the whole measured $Q$ range. The dashed lines are drawn to guide the eyes.

Understanding of these two short time dynamical behaviors, the phonon energy softening and phonon population enhancement, is important to clarify the essence of the intraprotein short time collective vibration and its relation to the protein enzymatic function. Since the protein dynamics is closely correlated to structure and environment [144], we are mainly concerned about the following questions: (1) how do the protein structures influence the phonon dispersion relation? In particular, whether the tertiary and secondary structures, which are the unique features of a protein, are the necessary condition for the onset of the phonon energy softening and population enhancement? And (2) what is the role of the hydration water in the short time intraprotein collective motion?

To access the above questions, we performed a series of IXS experiments on four different powders, i.e., hydrated native lysozyme, dry lysozyme, hydrated denatured lysozyme, and hydrated denatured $\alpha$-
Chymotrypsinogen A [145]. The hydration level $h$ for all the hydrated samples is 0.3. This value is well above $h = 0.2 \sim 0.25$, at which the polar groups in the protein surface are just completely saturated with water [127]. In this case the native lysozyme can gain enough mobility to function as enzyme.

Figure 4.6 (a) shows the dispersion relation of the phonon-like excitations for the native lysozyme sample with $h = 0.3$ at two temperatures (200 K and 300 K). One can find that within the $Q$ range of about 12 - 31 nm$^{-1}$, the phonon exhibits a substantial softening for the higher temperature (300 K) indicated by the lower energy. Meanwhile, the fractional area of the Brillouin peaks $A_s$ is significantly enhanced, as shown in Fig. 4.6 (b). These two phenomena are just the above-mentioned “phonon energy softening” and “phonon population enhancement”. In particular, the $Q$ range of 12 - 31 nm$^{-1}$ corresponds to the wavelength of about $l = 2 \sim 5.2$ Å ($l \sim 2\pi/Q$), which is comparable to the typical length scale of the spatial order of protein secondary structures ($\beta$-sheet average distance and $\alpha$-helix repeat and width) that is about 4 $\sim$ 5 Å. In order to study the relation between such “softening” and the protein structure, especially the protein secondary structure, we perform IXS measurements at $T = 200$ K and $300$ K on a hydrated lysozyme sample denatured by heating in alkali environment (denoted as den-Lys in the following part). For this sample, the amount of $\beta$-sheet increases, however, because the amount of $\alpha$-helix is greatly reduced, the overall amount of secondary structure is still largely reduced compared to the native counterpart [124]. This change of structure can also be reflected in the change of $S(Q)$ (shown in Fig. 4.6 (e)). Therefore if the “softening” is strongly correlated to the protein tertiary structure or the secondary structure, the dispersion relation of the den-Lys is expected to be quite different from that of the hydrated native lysozyme. The dispersion relation and the fractional area of the Brillouin peaks $A_s$ of den-Lys are shown in Fig. 4.6 (c) and (d) respectively. Remarkably, the den-Lys exhibits dispersion relations qualitatively similar to that of the native lysozyme, with an obvious phonon energy softening between 200 K and 300 K. The $T$-$Q$ dependence of $A_s$ is also similar to that of the native lysozyme. These results suggest that the native structure of protein is not the necessary condition for the “phonon energy softening” and the “phonon population enhancement”. Furthermore, the “phonon energy softening” seems not directly related to the secondary structure either, even the maximum softening appears at the $Q$ range
of ~ 13 - 21 nm\(^{-1}\), which corresponds to the wavelength of ~ 3 - 5 Å that is similar to the length scale of the spatial order of the protein secondary structures (4 - 5 Å).

Figure 4.6. (a) Dispersion relations of the phonon-like excitations and (b) fractional area of the Brillouin peaks \( A_s \) at \( T = 200 \) K and 300 K for hydrated native lysozyme. (c) Dispersion relations of the phonon-like excitations and (d) fractional area of the Brillouin peaks \( A_s \) at \( T = 200 \) K and 300 K for hydrated denatured lysozyme. (e) \( S(Q) \) curves (measured at \( T = 300 \) K) and three-dimensional graphical representations for the above two samples. The dashed lines are drawn to guide the eyes.

In order to verify the above statement, we performed another experiment on a denatured \( \alpha \)-Chymotrypsinogen A sample at \( h = 0.3 \) (denoted as den-\( \alpha \)-Ch). The denaturation is heat-induced in alkali
environment. In this sample, nearly all the tertiary structures and α-helices are removed, and only minor β-sheets are left [146]. Compared with den-Lys, den-α-Ch is even more unfolded. The dispersion relation of the phonon-like excitation, the fractional area of the Brillouin peaks $A_s$ and $S(Q)$ of this sample are shown in Fig. 4.7 (a), (b) and (c), respectively. One can find that the “phonon energy softening” and “phonon population enhancement” still exist. This result confirms our conclusion that these two phenomena are not directly related to the protein tertiary and secondary structures.

![Figure 4.7](image)

Figure 4.7. (a) Dispersion relations of the phonon-like excitations and (b) fractional area of the Brillouin peaks $A_s$ at $T = 200$ K and 300 K for hydrated denatured α-Chymotrypsinogen A. (c) $S(Q)$ curve (measured at $T = 300$ K) and three-dimensional graphical representation for this sample. The dashed lines are drawn to guide the eyes.

In previous paragraphs, we found that for lysozyme sample with native structure, the onset of its biological function is accompanied by the “phonon energy softening” and “phonon population enhancement”. Subsequently, we concluded that the short time intraprotein collective vibrational motions
are correlated to the establishment of enzymatic function in hydrated native lysozyme, in the sense that the samples with strong enzymatic function are “soft”, i.e., they exhibit low frequency and strong intraprotein collective vibrations. From this work, we show that the “phonon energy softening” and “phonon population enhancement” are not the sufficient condition for the enzymatic function of protein. This is similar to the situation of the so-called “protein dynamical transition” [124-126]. Proteins are dynamic systems with complicated energy landscape that can be divided into a number of tiers. A protein motion at a specific time scale can be considered as a sort of fluctuation in a particular tier of the energy landscape. Hence it is highly unlikely that one kind of short time protein motion can solely determine the enzymatic function of protein. In fact, a recent study shows that protein catalysis involves motions subjected to a wide range of time scales [111].

Figure 4.8. (a) Dispersion relations of the phonon-like excitations and (b) fractional area of the Brillouin peaks $A$ at $T = 200$ K and $300$ K for the dry lysozyme. The dashed lines are drawn to guide the eyes.

The weak dependence of the “phonon energy softening” on the tertiary and secondary structures indicates that this intriguing phenomenon may be closely related to the protein environment, i.e., the hydration water. Therefore a dry lysozyme sample was measured. Its dispersion relation and the fractional area of the Brillouin peaks $A$, are shown in Fig. 4.8 (a) and (b) respectively. From Fig. 4.8 (a) one can find that for the lysozyme without water, the “phonon energy softening” is considerably suppressed within $Q$
< 24 nm\(^{-1}\). This indicates that the hydration water plays a key role in the onset of the “phonon energy softening”. Figure 4.8 (b) shows that the “phonon population enhancement” still exists, since at high temperature it is easier to excite modes.

![Figure 4.8](image)

Figure 4.9. Dispersion relations of the phonon-like excitations for the hydrated lysozyme \((h = 0.3)\) and dry lysozyme at (a) \(T = 300\) K and (b) \(T = 200\) K. The dashed lines are drawn to guide the eyes.

In previous paragraphs, we show that hydration water sustains the “softness” at room temperature, in the sense that in certain \(Q\) range, the phonon energy of the sample at \(h = 0.3\) is smaller than that of the sample at \(h = 0.06\) (see Fig. 4.4). This phenomenon can also be seen in Fig. 4.9 (a): at \(T = 300\) K, the phonon energies of the hydrated lysozyme are smaller than those of the dry lysozyme within \(Q > 15\) nm\(^{-1}\).

In fact, similar phenomenon is also observed at \(T = 240\) K. However, the situation is different for the \(T = 200\) K case. From Fig. 4.9 (b) one can find that the phonon energies of the hydrated lysozyme are just as “hard” as those of the dry lysozyme. Such difference may be owing to a qualitative difference in the hydration water at \(T = 200\) K and \(T ≥ 240\) K. One candidate explanation is the dynamic crossover in the hydration water of protein [120]. As one passes the crossover temperature \(T_\alpha\) (~220 K) from below, the hydration water changes from the “strong” liquid, which is less fluid, to the “fragile” liquid behavior that is more fluid. Meanwhile, the relaxation time of the hydration water decreases significantly as \(T\) is
crossed from below. These phenomena may help the water to become a better “plasticizer” at the physiological temperature.

4.3 Dynamic Crossover in Protein Hydration Water

Proteins are dynamic systems that strongly couple with their environment [144]. Therefore, the study of the protein hydration water is crucial for the understanding of protein dynamics. One observation of the protein hydration water is the so-called dynamic crossover [120], which is referred to as a transition in the characteristic relaxation time of the hydration water molecule from an Arrhenius behavior at low temperatures to a super-Arrhenius behavior at high temperatures. At ambient pressure, the dynamic crossover takes place at \( T_c = 220 \text{ K} \) for hydrated lysozyme with \( h = 0.3 \). This phenomenon is of particular interest, partially because (i) the crossover temperature \( T_c \) of the hydration water is close to the transition temperature of the so-called protein dynamic transition (PDT) [118] \( T_D \) and thus it is conjectured that the PDT is induced by the dynamic crossover of the hydration water [120] and (ii) this crossover is interpreted as an anomalous feature of the structural relaxation of the hydration water and is ascribed to the existence of the high-density liquid and low-density liquid phases in the supercooled water [120]. To date, this phenomenon and its physical implications are still largely debated [147-152]. Doster \textit{et al.} [150] state that the crossover observed in Ref. [120] is due to numerical errors in the data analysis protocol and can be ruled out with an improved analysis method. Magazù \textit{et al.} [149] and Schirò \textit{et al.} [152] draw opposite conclusions on the role that the dynamic crossover plays in the onset of the PDT with resolution-dependent neutron scattering experiments. Swenson \textit{et al.} [147], Pawlus \textit{et al.} [148], and Fenimore \textit{et al.} [151] propose that the appearance of the dynamic crossover in the hydration water is due to the existence of two different relaxation processes, the structural relaxation and a secondary relaxation, rather than a qualitative change from an Arrhenius behavior to a super-Arrhenius behavior of the structural relaxation time.
In order to clarify some of the controversies over the dynamic crossover in the protein hydration water, we investigate the characteristic relaxation time of the hydration water of lysozyme at three hydration levels, \( h = 0.18, 0.30, \) and \( 0.45 \), with quasi-elastic neutron scattering [153]. The experiment was performed on the backscattering spectrometer BASIS [154] at the Spallation Neutron Source, Oak Ridge National Laboratory. In this study, for each hydration level, the spectra at two \( Q \) values, 0.5 and 0.9 Å\(^{-1}\), were measured. The energy window for data analysis is from -20 to 20 µeV and the energy resolution of the spectrometer is 3.4 µeV. For lysozyme, the hydration level of 0.2 - 0.25 (denoted by \( h_c \)) is critical: The hydrogen-bonding sites on the protein surface are just completely saturated with water at this hydration level [127]. We stress that \( h = 0.30 \) and \( 0.45 \), the two higher hydration levels studied, are well above \( h_c \). In contrast, the lowest hydration level \( h = 0.18 \) is slightly lower than \( h_c \).

The measured scattering intensity relevant to the water in equilibrium with the protein, \( I_{H_2O}(E) \), is obtained from the signal of the fully hydrogenated sample, \( I_{lys,H_2O}(E) \), subtracted by the signal corresponding to the sample hydrated with D\(_2\)O, \( I_{lys,D_2O}(E) \), at the same hydration level. Thus the obtained spectra were fit with the following equation:

\[
I_{H_2O}(E) = I_{lys,H_2O}(E) - I_{lys,D_2O}(E) = \{p\delta(E) + (1 - p)FT[F_s(t)]\} \otimes R(E) + B, \tag{4.3}
\]

Note that here we use a similar model as in Section 3.5. In the above equation, \( FT \) denotes a time Fourier transform, \( R(E) \) is the energy resolution function, \( \otimes \) is the convolution operator, \( p \) is the elastic fraction, and \( B \) is a background constant. \( F_s(t) \) is the self-intermediate scattering function (SISF). For supercooled water, the decay of \( F_s(t) \) has two steps [87]. The first step corresponds to some localized motions on sub-picosecond timescale. These motions are beyond the dynamic range of the spectrometer. The second step (long time) is highly nonexponential and is usually described by a stretched exponential decay form [87]. To account for these considerations, the experimental SISF is modeled as:

\[
F_s(t) \approx A(Q)\exp[-(t/\tau)^\beta], \tag{4.4}
\]

where \( A(Q) \) is the amplitude after the initial step, \( \tau \) is the characteristic relaxation time of the long-time relaxation, and \( \beta \) is the stretching exponent. The mean characteristic relaxation time \( \langle \tau \rangle \) is calculated
from $r$ and $\beta$ by $\langle \tau \rangle = \beta^{-1} \Gamma(\beta^{-1}) \tau$. In this study, $\beta$ is fixed to 0.5, just as in the analysis of Doster et al. [150] and of a previous work in Prof. Chen's group [120]. In addition, we have taken the elastic fraction $p$ to be of the form $p(Q) = p_0 A(Q)$, with $p_0$ constant. This is because both $p(Q)$ and $A(Q)$ are expected to have the form of a Debye-Waller factor. For each sample and a specific $Q$, the value of $p_0$ was obtained from the fit of the spectrum at 295 K, since the elastic fraction $p(Q)$ is most easily distinguished at the highest temperature. The analysis method introduced here has been successfully applied to study the single-particle dynamics of the deeply cooled water confined in silica matrice [107]. Figure 4.10 shows selected measured spectra for the samples with $h = 0.30$ and 0.45 along with the corresponding calculated curves fitted by Eq. 4.3. In principle, this method is same to the one Doster et al. used in Ref. [150], in which the authors highlight that no crossover is observed in the hydration water of protein in the QENS experiment. Thus a direct comparison between the results in this study and in Ref. [150] could be performed.

![Figure 4.10. QENS spectra of the hydration water at $T = 295$ K (a) and 235 K (b) for the sample with $h = 0.30$ (red squares) and 0.45 (green circles) at $Q = 0.5$ Å$^{-1}$. The fitted curves are denoted by solid lines. At 295 K, the protein hydration water at $h = 0.30$ is seen to relax more slowly than the one at $h = 0.45$. However, this ordering is reversed at 235 K.](image-url)
Figure 4.11. Arrhenius plot of the mean characteristic relaxation time $<\tau>$ at $Q = 0.5$ and 0.9 Å$^{-1}$ for the samples with $h = 0.30$ and 0.45. Panels (a) and (b) correspond to the samples with $h = 0.30$ and 0.45 respectively. The dynamic crossover takes place at about 220 K in the former case and at about 235 K in the latter case. The result of Doster et al. [150] for the C-phycocyanin (CPC) with $h = 0.3$ and at $Q = 1$ Å$^{-1}$ is also plotted in panel (a) and is denoted by (+) markers. Panel (c) shows the $<\tau>$ at $Q = 0.5$ Å$^{-1}$ for both the cases of $h = 0.30$ and 0.45. It is seen that the hydration water at $h = 0.45$ exhibits a more visible dynamic crossover than that at $h = 0.30$.

Figure 4.11 (a) shows the $<\tau>$ for the sample with $h = 0.30$. The higher-temperature data exhibit super-Arrhenius behavior, which can be fitted by the Vogel-Fulcher-Tammann (VFT) relation, $<\tau>$ =
The lower-temperature data exhibit Arrhenius behavior, which can be fitted by the Arrhenius relation, \( \langle \tau \rangle = \tau_0 \exp(\frac{E_a}{k_BT}) \). In this case, the crossing temperature of these two fits, i.e. the dynamic crossover temperature \( T_c \), is 220 K for all the measured \( Q \) values. As \( h \) increases to 0.45, the dynamic crossover shifts to 235 K, as shown in Fig. 4.11 (b). The parameters \( D \), \( T_0 \), and \( E_a \) for these two samples are listed in Table 4.1. It is seen that the parameters for the higher-temperature data, \( D \) and \( T_0 \), have strong \( h \) dependence. It leads to a significant difference in the curvature of the \( \langle \tau \rangle \) in the Arrhenius plot at higher temperatures as \( h \) increases from 0.30 to 0.45, as shown in Fig. 4.11 (c). Such a difference that the \( T \) dependence of \( \langle \tau \rangle \) with \( h = 0.45 \) exhibits stronger super-Arrhenius feature than that with \( h = 0.30 \) is also reflected in the measured spectra. As shown in Fig. 4.10, at \( T = 295 \) K, the hydration water at \( h = 0.45 \) relaxes faster than that at \( h = 0.30 \), indicated by a stronger inelastic scattering in the spectrum; however, as the temperature decreases to 235 K, this ordering is reversed: the hydration water at \( h = 0.45 \) relaxes slower than that at \( h = 0.30 \), indicated by a weaker inelastic scattering in the spectrum. For the samples with \( h = 0.30 \) and 0.45, the hydration level is well above the “monolayer” hydration level \( h_c \), thus the hydration water molecules are abundant enough to form an open tetrahedral structure. Under this circumstance, a molecule is likely to be trapped in a cage consisting of its neighboring molecules. The translational diffusion jump of this molecule requires “cage breaking” that is associated with simultaneous breaking of several hydrogen bonds. As \( T \) decreases, the local order surrounding a molecule is enhanced, which leads to a significant increase of the structural relaxation time [87]. Subsequently, this collective relaxation process exhibits non-Arrhenius \( T \) dependence. As \( h \) increases, the “cage effect” is enhanced, which causes the \( T \) dependence of \( \langle \tau \rangle \) to deviate from the Arrhenius behavior more significantly. In contrast to \( D \) and \( T_0 \), the activation energy \( E_a \) for the lower-temperature data is insensitive to \( h \), as shown in Table 4.1. In addition, we plot the neutron scattering result for the hydration water of deuterated C-phycocyanin (CPC) with \( h = 0.3 \) and at \( Q = 1 \) Å⁻¹ in Fig. 4.11 (a) [150]. Not surprisingly, the data agree well with our data for the sample with \( h = 0.30 \) and at \( Q = 0.9 \) Å⁻¹, due to the similar \( h, Q \) and analysis method in these two cases. With the result of CPC, Doster et al. [150] conclude that no dynamic crossover happens. However, our analysis confirms that the dynamic crossover takes place when the
hydration water is sufficient enough, and it becomes more visible as $h$ increases, due to the strong $h$
dependence of the higher-temperature data. In fact, similar phenomenon is also observed in the water
confined in nanoporous silica material MCM-41. Faraone et al. [24] show that as the diameter of the
confining pore increases from 14 to 18 Å (thus the water content also increases), $D$ changes from 4.62 to
1.47, and $T_0$ changes from 170 to 200 K. Such $h$ dependences of $D$ and $T_0$ exhibit good agreement to the
results of the protein hydration water shown in Table 4.1. As a result, the $T_0$ of the water confined in
MCM-41 increases from 222 to 225 K as the size of the confining pore increases, which exhibits same
trend as the case of the protein hydration water.

<table>
<thead>
<tr>
<th>$h$ (g/g)</th>
<th>$Q$ (Å⁻¹)</th>
<th>$D$</th>
<th>$T_0$ (K)</th>
<th>$E_a$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>0.5</td>
<td>4.96</td>
<td>147.99</td>
<td>9.20</td>
</tr>
<tr>
<td>0.30</td>
<td>0.9</td>
<td>9.98</td>
<td>122.64</td>
<td>9.26</td>
</tr>
<tr>
<td>0.45</td>
<td>0.5</td>
<td>1.41</td>
<td>201.24</td>
<td>8.93</td>
</tr>
<tr>
<td>0.45</td>
<td>0.9</td>
<td>1.03</td>
<td>206.78</td>
<td>9.85</td>
</tr>
</tbody>
</table>

Table 4.1. Parameters in the VFT relation and the Arrhenius relation for $h = 0.30$ and 0.45.

For the hydration water at $h = 0.18$, its Arrhenius plot of the $<c>$ is shown in Fig. 4.12. In this case,
no significant crossover is observed, the $T$ dependence of $<c>$ can be described by the Arrhenius relation
in the whole range of measured temperature. The activation energies $E_a$ at $Q = 0.5$, 0.7 and 0.9 Å⁻¹,
obtained from the fit with the Arrhenius relation, are 9.31, 10.15 and 10.14 kcal/mol respectively. As
mentioned before, the hydration water of this sample merely saturates most of the polar groups on the
protein surface. The chance to form an open tetrahedral structure is little. Therefore, it is likely that the
water-surface interaction, rather than the water-water interaction, dominates the relaxation process. In fact,
the disappearance of the dynamic crossover and the super-Arrhenius behavior at higher temperatures are
also found in the hydration water of inorganic materials [107, 155, 156]. Interestingly, the activation
energies for this sample are similar to those for the lower-temperature data of the samples with $h = 0.30$
and 0.45. In addition, the activation energies obtained here, which are around 9 - 10 kcal/mol, agree well
to the activation energies of the hydration water for many other soft and biological materials [157]. The weak hydration level and environmental dependencies of $E_a$ suggest that the corresponding motions are local-like [157].

Figure 4.12. Arrhenius plot of the mean characteristic relaxation time $\langle \tau \rangle$ at $Q = 0.5, 0.9 \text{ Å}^{-1}$ for the sample with $h = 0.18$. In this case, the $T$ dependence of $\langle \tau \rangle$ can be described with Arrhenius relation in the whole range of measured temperature.

Owing to the similarity of $T_s$ and $T_D$ for the protein with $h = 0.3$, in a previous work [120], the authors tentatively ascribed the onset of the PDT to the dynamic crossover of the protein hydration water. This conjecture is under debate [43, 149, 152, 158]. It is well known that most of proteins only function with sufficient hydration water. Therefore, to clarify the relation between the PDT and the dynamic crossover in hydration water is important in learning how protein works. Roh et al. investigated the PDT of lysozyme at $h = 0.18, 0.30$ and $0.45$ (Fig. 2 in Ref. [129]). Thus a direct comparison between the PDT and the dynamic crossover in protein hydration water can be performed. The common features of the PDT and the dynamic crossover can be summarized as: (i) both of these two phenomena appear at $h = 0.30$ and $0.45$, and are strongly suppressed at $h = 0.18$; (ii) both of them are enhanced as $h$ increases. However, the $h$ dependences of $T_D$ and $T_s$ are not similar to each other. According to Roh et al. [129], the values of $T_D$ at $h = 0.30$ and $0.45$ are both around 200 K, which is substantially different from the results of $T_s$, shown
in this study. Moreover, Paciaroni et al. [128] report that $T_D$ even decreases as $h$ increases. To account for these results, we conclude that the PDT is not directly induced by the dynamic crossover in the protein hydration water.

In summary, we investigate the single-particle dynamics of the protein hydration water at different hydration levels. The dynamic crossover phenomenon appears when $h$ is higher than the “monolayer” hydration level, $h_c = 0.2 - 0.25$, and becomes more visible as $h$ increases in the measured range. It disappears when $h$ is slightly lower than $h_c$, and in this case $\tau$ only exhibits Arrhenius behavior in the whole range of measured temperature. The higher-temperature data for the samples with $h = 0.30$ and 0.45, which exhibit super-Arrhenius behavior, are sensitive to $h$. On the contrary, the lower-temperature data, which exhibit Arrhenius behavior, are relatively insensitive to $h$ and display local-like characteristic. These results highlight the importance of the tetrahedral hydrogen-bond network in the dynamics of the hydration water. In addition, the crossover temperature exhibits different $h$ dependence from that of the protein dynamic transition (PDT) temperature. This difference clearly shows that the PDT is not directly induced by the dynamic crossover in the hydration water.
Chapter 5  Wrap-Up

5.1 Confined Water, Aqueous Solutions and Bulk Water

This thesis describes our contributions to the study of the phase behavior of the deeply cooled water confined in MCM-41 with neutron scattering technique. We first consider the average density of the confined water with elastic neutron scattering at different temperatures and pressures. By detecting the density hysteresis, we establish the phase diagram of the liquid-liquid transition in the deeply cooled region of the confined water, which is shown as follows. Note that, in Fig. 2.5, we show that the critical point is at $P = 1.29 \pm 0.34$ kbar, $T = 213 \pm 3$ K. With an additional experiment, we find that effective hysteresis still exists at 1.29 kbar. Thus the position of the critical point is updated to $P = 1.12 \pm 0.17$ kbar, $T = 215 \pm 1$ K in the following figure.

![Phase diagram of the liquid-liquid transition in the confined heavy water.](image)

Figure 5.1. Phase diagram of the liquid-liquid transition in the confined heavy water. The solid line, dashed line and pentacle denote the liquid-liquid transition line, the Widom line and the LLCP, respectively.

The average density is a time-independent equilibrium property of the system. The existence of the two phases leads to different local structures and different hydrogen-bond strengths. Therefore, it must
have impact on the dynamic properties of the system. Parallel to the density measurement with static neutron scattering, we also test the phase diagram shown above by measuring various dynamic properties of the system with dynamic neutron scattering. In this thesis, we measure three dynamic properties of the confined water: the librational band in the vibrational density of state, the boson peak and the structural relaxation. The time scales of these three motions cover a broad range from $10^{-2}$ picosecond to hundreds of nanoseconds. A sketch of the time dependence of the correlation function $I(t)$ of the deeply cooled water is shown as follows. In neutron scattering studies, $I(t)$ is usually represented by the self-intermediate scattering function that is expressed by

$$I(Q, t) = \frac{1}{N} \sum_{i=1}^{N} \exp[-i \cdot \vec{r}_i(0)] \exp[i \cdot \vec{Q} \cdot \vec{r}_i(t)],$$

where $N$ is the total number of hydrogen atoms, $\vec{r}_i(t)$ is the position of hydrogen atom $i$ at time $t$.

![Sketch of the time dependence of the correlation function $I(t)$ of the deeply cooled water.](image)

Figure 5.2. Sketch of the time dependence of the correlation function $I(t)$ of the deeply cooled water.

Among these three motions, the librational motion is the fastest and most local. It reflects the strength of the hydrogen bond connecting a water molecule and its nearest neighbors. The measurement suggests that the hydrogen bond between the central water molecule and its first shell in HDL is slightly weaker than that in LDL. This difference is similar to the difference between LDA and HDA.

Boson peak has a time scale of $10^{-4}$ picosecond. It reflects the collective translational vibration in the system. Its shape depends on the density and the local structure of the system. We find that (1) in the one-
phase region, the emergence of boson peak is correlated to the Widom line determined by dynamic crossover measurement; and (2) in the two-phase region, the shape of the boson peak, including the mean frequency, the height and the width, exhibits a jump between 3 and 4 kbar, which indicates an abrupt change in the density of water between 3 and 4 kbar. In addition, the existence of two different master curves of boson peak strongly suggests the existence of two structurally distinct phases, the LDL and the HDL.

The structural relaxation of the confined water has a time scale from $10^1$ picosecond at room temperature to more than $10^3$ picosecond at temperatures below 200 K. It is related to the configuration of the hydrogen-bond network in water. The measurement on the relaxation process of the confined water suggests that the LDL has a stronger (or more ordered) local tetrahedral hydrogen-bond network than the HDL. This result is consistent with the study on the structures of LDL and HDL [29].

Our density measurements and dynamic measurements give a coherent picture on the phase behavior of the deeply cooled water confined in MCM-41. According to these results, we conclude that we provide clear evidence for the existence of the liquid-liquid transition in this confined water system.

Besides confinement, other methods have been used to enter the "no man's land" of the liquid water. It is found that by adding additives, the homogeneous nucleation temperature can be lowered [159, 160]. So that one may get a chance to access the temperatures at which the liquid-liquid transition takes place. Mishima studies the phase behavior of the dilute LiCl solution with thermodynamic method and X-ray and Raman scattering [161]. He finds that when an emulsified 4.8 mol % LiCl-H$_2$O solution was cooled under a pressure of 0.35 or 0.45 GPa and decompressed to 0.1 GPa at 142 K (slightly above its glass transition temperature 140 K at 0.1 GPa), its volume increased suddenly. With the results from X-ray and Raman scattering experiments, he assigns this phenomenon as an appearance of the low-density amorphous ice in the liquid solution. This appearance corresponds to the high-to-low-density polyamorphic transition of pure H$_2$O.

Glycerol and other organic solutes have also been used as anti-freezing agent to enter the "no man's land" [40, 41, 162]. Suzuki and Mishima examine the volume change of the glycerol solution at different
pressures, temperatures and concentrations [162]. They observe a liquid-liquid transition with its critical
point at 0.12 - 0.15 mole fraction, 0.03 - 0.05 GPa, and 150 K in such system. Murata and Tanaka study
the kinetic process in the glycerol solution [41]. They find two phases that differ in density, structure,
refractive index, etc. at ambient pressure. These two phases may correspond to the LDL and HDL in such
system.

Experimental detection of the LDL and HDL in pure bulk water is even more difficult. In the early
stage, researchers studied the structure of the water at ambient pressure as a function of temperature with
X-ray and neutron diffraction [163, 164]. These works show that the water molecule is surrounded by a
random tetrahedral hydrogen-bond structure. This open structure is very different from the local structure
of the simple liquid, and causes the thermodynamic anomalies. Nevertheless, these studies are far from
enough for proving the existence of HDL and LDL, because they do not give any information at higher
pressures. In 1995, Bellissent-Funel and Bosio investigated the structure of liquid D2O at pressures up to
6 kbar and in a range of temperatures from 326 down to 208 K [165]. This work shows that the
temperature dependence of the local structure of water changes significantly from ambient pressure to
pressures higher than 2600 bar. This result is consistent to the existence of a LDL-to-HDL transition at
around 2 kbar. Soper and Ricci made another trial in 2000. With wide angle neutron scattering technique,
they measure the structure of the bulk pure water at 268 K and under different pressures [29]. They find
that as pressure increases, the local structure of water has a continuous transformation from a low-density
form, with an open, hydrogen-bonded tetrahedral structure, to a high-density form of water, with
nontetrahedral O-O-O angles and a collapsed second coordination shell. By an extrapolating method, they
obtain the structures of LDL and HDL. These works are a very important because they show that in the
bulk pure water it is possible to exist two structurally different liquid phases. However, they are still
outside the “no man’s land”.

In 2014, Nilsson and his collaborators reported data on the structure of liquid water well below the
homogeneous nucleation temperature (232 K at ambient pressure) [166]. They prepare a jet of water
droplets with the length scale of 10^{-5} m and measure their structures with ultra-fast X-ray diffraction
technique. They find that at the temperature of 227 K, the local structure of liquid water is drastically changed from the local structure of water at ambient conditions. In this case, water is almost a tetrahedrally structured liquid, which is the signature of the LDL phase. It is regrettable that they did not provide data at higher pressures (as pointed out by Soper, this is very difficult [167]). Nevertheless, it is still a breakthrough because these researchers lower the temperature limit for liquid water by 23 K from the previous record [168] and get the data on the structure of the bulk liquid water in the “no man’s land”.

The concepts of HDL and LDL, after being introduced by Stanley and his collaborators, attracted a great deal of attention. The idea that two structurally different phases can exist in a one-component liquid is profound. In last two decades, researchers from different disciplines, using computer simulations and experimental methods, attack the “no man’s land” and try to find clues for the liquid-liquid transition. Thought a unified opinion still lacks, all of these efforts represent steps towards an ultimate understanding of the unique behavior of liquid water.

5.2 Perspective Future Research

The study of the phase behavior of the deeply cooled confined water is still not adequate. In this section, I would like to discuss some possible future works.

The phase diagram of the deeply cooled confined water shown in Fig. 5.1 is significantly different from the conjectured phase diagram of the bulk water [92]. The pressure of the liquid-liquid transition line of the confined water is higher than that of the bulk water by about 1 kbar. The reason might be attributed to the capillary effect due to the confinement in pores of cylindrical geometry. For the fluid confined in a hydrophilic tube, the liquid-vapor surface forms a meniscus, which will lead to a pressure difference across this surface. Here we use the Young-Laplace equation [169] to give a rough estimation of the pressure difference for our case: when the tube is sufficiently narrow, the pressure difference can be expressed as $P = 2\gamma\cos\theta/R$. Where $\gamma$ is the surface tension of the fluid, $\theta$ is the contact angle and $R$ is the radius of the tube. With the values of $\gamma$ and $\theta$ at room temperature, the pressure difference for the
nanopores of MCM-41 is estimated to be in the order of 1000 bars, which is qualitatively consistent with the pressure difference between the results of the bulk water and the confined water. Note that, Young-Laplace equation cannot describe the liquid confined in nanopores accurately. Further studies on the pressure effect of the cylindrical nanopore are necessary. These studies may provide a link between the bulk water and confined water.

In this thesis, we perform studies on the density and dynamic properties of the confined water. The study on the structure of the confined water lacks. This kind of study can be performed with wide angle neutron scattering technique. By measuring the $S(Q)$ of the confined water at the LDL region and HDL region in the phase diagram shown in Fig. 5.1, one can get direct information on the structural transformation as the average density changes in the confined water. Particularly, Mancinelli et al. point out that the structure of the confined water is rather complicated [170]. Therefore, such kind of experiment is needed to provide structural evidence on the liquid-liquid transition in the confined water and to lessen the relevant debates.

Here I quote the verse by Swinburne as the end of this thesis:

\begin{quotation}
That even the weariest river,
\end{quotation}

\begin{quotation}
Winds somewhere safe to sea.
\end{quotation}
Appendix  A List of Publications


Bibliography


[20] The pore sizes determined by different BJH machine can have slight difference. In addition, the pore size determined by BJH method may underestimate a more geometric definition of the pore diameter.

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