

Probing Collective Motions and Hydration Dynamics of Biomolecules by a Wide Range Dielectric Spectroscopy

Ali Charkhesht

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Vinh Q. Nguyen, Chair
Giti A. Khodaparast
Hans Robinson
Michel Pleimling

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ABSTRACT

Studying dynamics of proteins in their biological milieu such as water is interesting because of their strong absorption in the terahertz range that contain information on their global and sub-global collective vibrational modes (conformational dynamics) and global dynamical correlations among solvent water molecules and proteins. In addition, water molecules dynamics within protein solvation layers play a major role in enzyme activity. However, due to the strong absorption of water in the gigahertz-to-terahertz frequencies, it is challenging to study the properties of the solvent dynamics as well as the conformational changes of protein in water. In response, we have developed a highly sensitive megahertz-to-terahertz dielectric spectroscopy system to probe the hydration shells as well as large-scale dynamics of these biomolecules. Thereby, we have deduced the conformation flexibility of proteins and compare the hydration dynamics around proteins to understand the effects of surface-mediated solvent dynamics, relationships among different measures of interfacial solvent dynamics, and protein-mediated solvent dynamics based on the complex dielectric response from 50 MHz up to 2 THz by using the system we developed. Comparing these assets of various proteins in different classes helps us shed light on the macromolecular dynamics in a biologically relevant water environment.

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GENERAL AUDIENCE ABSTRACT

Proteins are complicated biomolecules that exist in all living creatures and they are, mostly, involved in building up structures and cell functions in various biological systems. Not only their existence but also their complex movements and dynamics are vital to cell functions in living beings. Until recently, their chemical functions and dynamics have been extremely challenging to investigate and track in their native environments. Thanks to various efforts by researchers all over the world to learn more about their convoluted behavior, new techniques have arisen to study these properties. We, as a part of this community, have been able to develop highly sensitive megahertz-to-terahertz dielectric spectroscopy system to probe proteins and other biomolecules dynamics in picosecond to microsecond range. Using our benchmark system, we have been able to map the detailed dynamical properties of biomolecules as well as their exclusive hydration shell characterizations. In this work, we gathered details about three well-known proteins and biomolecules by studying their dielectric responses. Thus, we have been able to discuss the movements, relaxation processes and hydration shell properties of these molecules in liquid water as their basic native environment.

To My Beloved Parents,

Naiyer Tahaeinazhad

and

Abdolali Charkhesht

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Chapter 1

Introduction

1.1. Terahertz Science and History

The terahertz (THz) spectral region of the electromagnetic spectrum lies in the gap between the microwave and infrared region with a frequency range of 0.1 THz to 10 THz (0.3 mm – 30 μ m). This region is also known as the submillimeter band or the far-infrared (FIR) region depending on which electronic techniques or optical approach you subscribe to, respectively. However, the term “terahertz,” which was coined around 1974, is most commonly used for these radiations [1-3].

This region has been gaining attention since the 1920s, especially in different aspects of spectroscopy. The terahertz range has been widely used in astronomy, atmospheric studies, chemistry, biophysics, and condensed matter physics to describe the absorption and transmission properties of materials. However, scientific research in this band has been limited due to the excessive absorption properties of water molecules that stem from their rotational and vibrational modes. These modes occur over most of the frequency range, limiting the atmospheric propagation path of THz rays. Thus, designing a suitable THz operating system, as well as sources, detectors, waveguides, etc... has been the primary challenge for researchers in this field over the last few decades. Depending on the subject to be investigated, there have been numerous approaches to solving practical difficulties of terahertz radiations in order to offer high resolution spectroscopy techniques that lie between traditional microwave and optical technologies. Recent advances in THz sources and detectors introduced the potential to expand this research to new applications [4-7].

In the 1950s, thermal sources such as heated solids and plasma discharge lamps were reliable sources from the IR range to higher frequencies of THz [8]. Later in 1970s, electrically and optically excited gas lasers were used as sources to produce radiation above 1 THz [9, 10]. Over the past decade or so, quantum cascade lasers (QCLs), p-Ge lasers (THz lasers) and free-electron lasers (FELs) have become the most dependable sources to carry out experiments in the THz region [11-13]. Developments in sub-picosecond and femtosecond mode-locked lasers have introduced novel techniques in THz physics leading to THz time domain spectrometers (TTDs) [14, 15].

In addition to optical tools in THz region, recent microwave technology has introduced promising methods in terahertz engineering and science. Extending frequency ranges from microwave to THz using harmonic generators as consistent sources is one of the main keys to this approach. Terahertz frequency domain spectroscopy (TFDs), which is based on this technique, opened a new window in the terahertz spectroscopy field. Multiplying frequencies from high-power microwave sources into the THz frequency range would offer high power, coherent, continuous waves (cw) in this region that can cover mid gigahertz to low terahertz frequencies with better output power (~ 150 mW). For instance, using a standard microwave generator (e.g. vector network analyzer), feeding frequency extenders in different bands could be assumed as a dependable THz source. It should be kept in mind that the output power could be inconsistent on different extender frequency bands and that it drops with number of multiplications n and frequency ν while phase noise may increase by $20 \log(n)$ [16, 17].

1.2. Applications

The fact that THz can fill the gap between microwave and infrared frequency regions has made THz radiation a driving force in merging electronic and optical technologies. Thus, this frequency band has become a new frontier of sorts for electromagnetic research within the last several years. Its remarkable radiation properties—such as penetration through cover materials with minor attenuation, better image resolution when compared to microwaves, and the material characterization potential in the frequency range, have made THz radiation an interesting and useful tool in many fields [18].

The photon energy of 1.2 to 12.4 meV (1mm to 100 μ m) is equivalent to black body radiation of 14 to 140 K, which makes the THz band a great choice for astronomers to study spectrum of an interstellar dust cloud [19]. Moreover, THz measurements are applied in the areas of plasma fusion diagnostics. The studies on temperature of plasma core, characterization of electron temperature fluctuations in the core, and gas spectroscopy can be achieved using suitable terahertz spectrometers [18, 20, 21]. Additionally, the radar

industry [22], terahertz imaging [23], and communication science [24], are other examples of growing fields in the terahertz community.

Even medical researchers have become interested in this band. Because of its shorter wavelength, THz technology presents enhanced spatial resolution in imaging. Therefore, next to X-ray imaging, magnetic resonance imaging (MRI) and ultrasonography, terahertz spectroscopy has received increasing attention in cancer detection. Thanks to extensive investigations of proteins and biomolecules in this frequency range, medical science is currently able to find new aspects of drug developments and improvements in cancer therapy [25, 26].

1.3. Terahertz Spectroscopy of Biomaterials

Terahertz technologies are widely used in studies of molecular systems. They have been practiced in investigations of the dielectric response of biomolecular systems in different tissues, environments, and organisms. Many biomolecules present specific absorption lines and dielectric responses to electromagnetic probe waves, in frequency range 0.1 to 5 THz, that are known as spectral “fingerprints.” This makes THz rays one of the best electromagnetic sources to probe these fingerprints in biomolecules characterization.

Proteins, a popular candidate for biomolecular studies, have their own special chemical functions, dynamics, and motions. Their dynamics are most directly influenced by the transitions between different states like enzymatic activities and bonding and un-bonding of proteins. These dynamical properties can be detected with collective vibrations corresponding to conformational changes, rotational motion, tumbling, etc... that are unique for each biomolecule. The hinging activity of lysozyme, dynamics of heme group of myoglobin molecules in storing oxygen, twisting and deformation of the DNA double-helix structure, are some prominent examples of biomolecules dynamics that are mostly in the picosecond to nanosecond timescale. Therefore, terahertz radiations from MHz to THz provide unique opportunities to probe this response timescale that makes them, by far, the best tool to be utilized as remote sensing probe in this matter.

A challenging problem is the utilization of tracking these type of dynamics in a natural environment for biomolecules which would involve water molecules. The dissolved biomolecules exhibit tumbling, low frequency collective vibrational, and rotational modes that have a tendency to be marked as their fingerprints. Bearing in mind that water molecules have huge absorption in this band, as shown in Figure 1.1, their absorption can be a blessing or a curse depending on the subject of study. Air moisture is definitely a nuisance in atmospheric studies and telecommunication methods that reduces

the propagation range of terahertz radiations, however it can be a miracle in biological studies where it can help track down biomolecules dynamics using water dynamics as sensitive probe element.

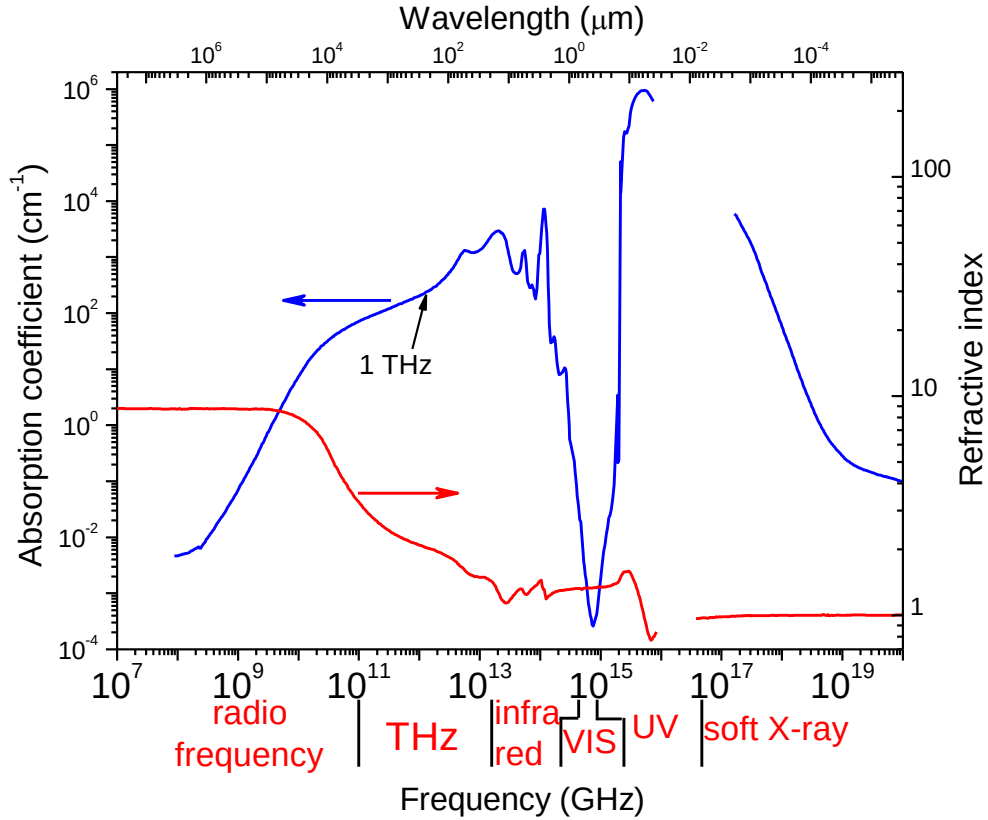


Figure 1.1: Absorption coefficient and reflective index of water changing with the frequency. This graph shows how water absorption and refractive index variation is very high in THz region comparing to other bands [27, 28].

Solvated biomolecules in liquid water usually form hydration shells around themselves by interacting with water molecules via hydrogen bonds. Depending on the Coulombic potential, they attract the polar water molecules from water pool into their hydration shell. Hydration water molecules are diverse in Coulombic potential depending on distance from solute and its active or charged sides. Thus, their dielectric responses to electromagnetic wave penetrating through the solution would be noticeably different than water molecules in bulk water pool.

Considering a protein as a typical solute, we can define three different types of water molecules with different relaxation dynamics: tightly bound in a hydration shell,

loosely bound around a shell in a weaker Coulombic potential, and bulk water molecules in a water pool. Proteins are large biomolecules with higher molecular weight, and are thus significantly heavier than water molecules. Consequently, their dynamics and relaxation time would be much slower than free water molecules in the KHz to MHz range that would be related to tumbling and slow molecular rotations. The very first layers of water molecules around the protein sitting on and interacting with the surface of the protein would have less sensitivity than free water. So, they will respond to probe radiations faster than proteins but slower than bulk waters. The other water molecules outside but close to hydration shell would have faster dynamics than tight water because their dipole moments interact less with protein molecules. However, they are not as free as bulk water molecules that can rotate and align their electric dipole moment direction with electrical field of incoming wave. Bulk water molecules, which are low in concentration of protein solutions, have the strongest absorption and dielectric response to probe waves around 20GHz. Their dynamics are usually very noticeable in dielectric spectrum. The characteristics of these dielectric responses are unique for each biomolecule and they can be used to clarify their nature in such a native environment as liquid water solutions. It is worth nothing that water molecules absorption limits applications of THz radiations, however they most certainly have an advantage in biomolecular spectroscopy research.

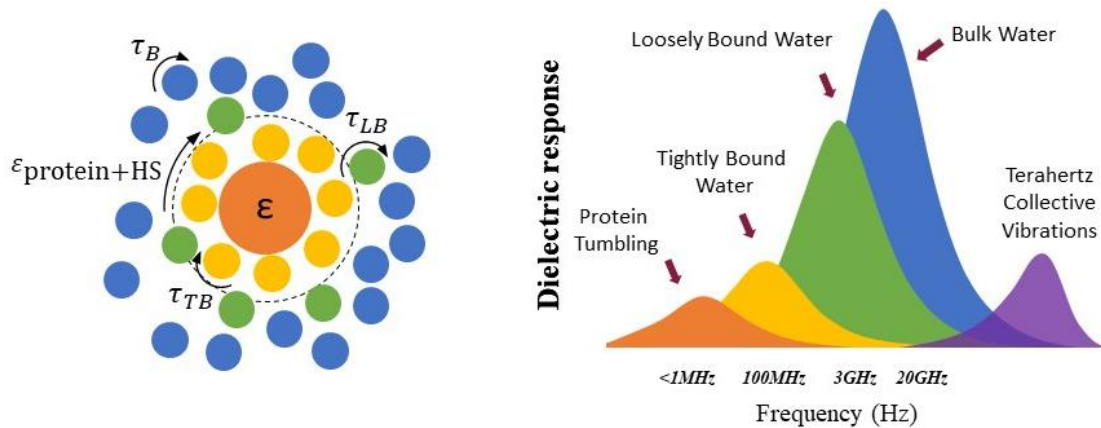


Figure 1.2: Schematic representation of dielectric response of a protein in water; This picture shows how different type of water molecules can be formed around a solute with various response time and dielectric value [the plot is developed by "Ali Charkhesht"].

The higher frequency part of Figure 1.2 is closely related to the collective vibrations of protein with hydration shell. The dielectric response in this region offers delegate information about special chemical functions of different proteins such as breathing dynamics of protein including hydration shell, hinging activities, or their enzyme activities. It should be mentioned that this picture is just a schematic imagination of protein water

molecules response to MHz to THz band radiations. The very detailed pictures for different types of proteins are shown in following chapters.

THz sources are bringing scientists even closer to understanding macroscale dynamical properties of biomaterials. Their penetration potential as well as their remote sensing aptitude makes them a biologically innocuous compatible contestant against X-rays and NMR (that may cause ionizing effect and needs a labeling material, respectively) for biomolecules characterization.

1.4. Overview

In this thesis, we describe our applications of the terahertz frequency domain spectroscopy to perform various studies on biomolecules. We have designed, developed, and implemented a very precise and accurate TFDs within our lab in order to characterize biomolecules in liquids by tracking their molecular dynamics and hydration shell properties. Additionally, the open-ended coax probe technique is used to extend the coverage spectra of the system. Using these techniques, we have been able to cover a very wide range of frequencies from 5 GHz to 2 THz in order to map the precise dynamical properties of biomolecules. The general idea is measuring the intensity changes and phase shifts of electromagnetic wave propagating through the biomaterials. These will give us the ability to calculate absorption coefficient and refractive index of subjects under study. Consequently, we have been capable of computing complex dielectric responses of solutions as well as biomolecules themselves. An adult human body consists of ~70% water, this would give us enough motivation to understand biomolecules dynamics in liquid solutions with water as a buffer. Although, water has a massive absorption coefficient in THz range of frequency, we could develop a powerful megahertz (MHz) to terahertz (THz) spectroscopy system to investigate real time dynamics of biomolecules, their hydration water behavior and bulk water responses.

Chapter 2 provides general information about our experimental setups, open-ended probe and terahertz frequency domain (TFDs) systems. More information on the theoretical and experimental aspects of our homebuilt TFDs setup is in Chapter 3 along with detailed information about the variable length sample cell, dynamic range of the system, terahertz frequency domain setup, frequency extenders, and vector network analyzer. In chapters 4, 5, and 6, dynamics of three well-known biomolecules, BSA, micelle, and glycerol, are investigated using our spectroscopy systems. The hydration shell dynamics, collective vibrations, rotational dynamics as tumbling motion of proteins, and biomolecules have been subjects of study.

In addition to biomaterial studies, appendix A reports an investigation into dynamics of phonon modes and their effect in structure transformations of Aurivillius materials. We have used our TFDs system to observe absorption and refractive index

variations as well as complex dielectric response of $\text{Bi}_4\text{Ti}_3\text{O}_{12}$ (BiT), as a model system to understand phonon modes related to phase transitions in different temperatures. Using this approach, we have proved that phonon softening is under influence of the anharmonicity in Bi-O bonds, and that Bi cations have a significant role in the emergence of ferroelectricity.

Chapter 2

Experimental Techniques

This chapter describes the experimental methods and setups applied to the wide frequency range of megahertz to terahertz of electromagnetic spectrum. These methods serve to aid in the investigation of the collective motions and hydration dynamics of biomolecules. Two major techniques: open-end probe and terahertz frequency domain spectroscopy (TFDs), have been used to carry out experiments from 50 MHz up to 1.12 THz.

2.1. Open-end Probe

To study the relaxation processes in lower frequency THz bands, microwave techniques are a suitable choice to carry out experiments in the frequency range of 10 MHz to 50 GHz (6 mm to 30 mm). We were able to implement an enhanced open-end probe using the Agilent 85070E dielectric probe kit and a vector network analyzer (Agilent PNA N5225A) as microwave sources. Three standard calibration processes, air, short, and a known material, in our case water, are needed for this one-port measurement technique. Using Agilent software, provided with a probe kit, one can measure the complex dielectric response of the material under test, including the real (dielectric dispersion), $\epsilon'_{\text{sol}}(\nu)$, and the imaginary (dielectric loss), $\epsilon''_{\text{sol}}(\nu)$, components with an accuracy of $\Delta\epsilon/\epsilon = 0.05$ in the frequency range.

Figure 2.1 shows the open-end probe setup that has been used to study various biomolecular solutions in liquid form. An anodized aluminum sample holder and Lakeshore 336 temperature controller with an accuracy of ± 0.02 °C are used to complete measurements on liquid samples. We have used a Y-axis stage to minimize human errors in both calibration processes and measurements. With this experimental setup, it is possible

to study the relaxation processes of water molecules, hydration water and biomolecules not lower than 10 MHz in wide range of temperatures from ~ 0 °C up to 90 °C.

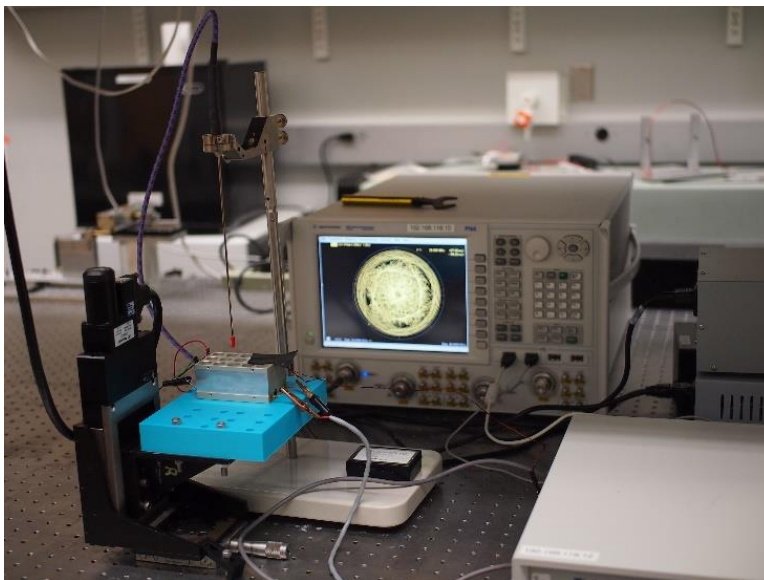


Figure 2.1: Open-end probe setup for carrying out low-frequency measurements on aqueous solutions. Using this setup, we can directly measure real part and imaginary parts of dielectric constant from 10 MHz up to 50 GHz.

2.2. Terahertz Frequency Domain Spectroscopy

To extend our studies on water and solvated biomolecules behavior in the picosecond dynamical range, we have established a benchmark GHz to THz dielectric frequency domain spectroscopy system. Using our custom-built setup, we have been able to cover the spectral range of 5 GHz to 1.12 THz (0.268 mm to 60 mm). Our spectrometer consists of two main systems: a commercial vector network analyzer (VNA) and harmonic frequency extenders provided by the Virginia Diode system (VDI). The VNA (Agilent PNA N5225A) as microwave sources covers the range of 10 MHz to 50GHz and VDI system supports to extend this range to 65 GHz to 1.12 THz. Using this setup, an output power up to 20 mW for coherent and continuous wave (cw) THz radiation is available with bandwidths from 1 Hz to 18 Hz. Thanks to the high dynamic range of 110 dB of our spectrometer, we could significantly improve signal-to-noise and spectral resolution for this wide range of frequencies.

We have been able to continuously measure the intensity change (ΔI) and phase shift ($\Delta\phi$) of wave propagating through materials using this system. This gives us the

opportunity to calculate the absorption coefficient (α) and refractive index (n) of various types of solvated biomolecules as well as water molecules. Consequently, mapping of complex dielectric response ϵ^* of different elements that would allow us to study both the relaxational (rotational) and translational processes of waters and biomolecules has been achieved using this novel setup.

Our TFDs system, which we used to track the picosecond dynamical time scale of solvated biomolecules, water molecules, and hydration water properties is shown in Figure 2.2. Samples under test are kept in variable path-length cell attached to translation stage, which provides 1 nm minimum incremental motion, to scan the entire frequency range for different thicknesses of biomaterial solutions. Thereby, we could have a precise picture of diverse elements in solution despite of high absorption of water molecules. A Lakeshore 336 temperature controller, Peltier cooler plates from Custom Thermoelectric (12711-5L31-03CK), and high power resistors, are used to control and adjust temperature fluctuations of samples. The specific theoretical and experimental information of this system is discussed in detail in Chapter 3.

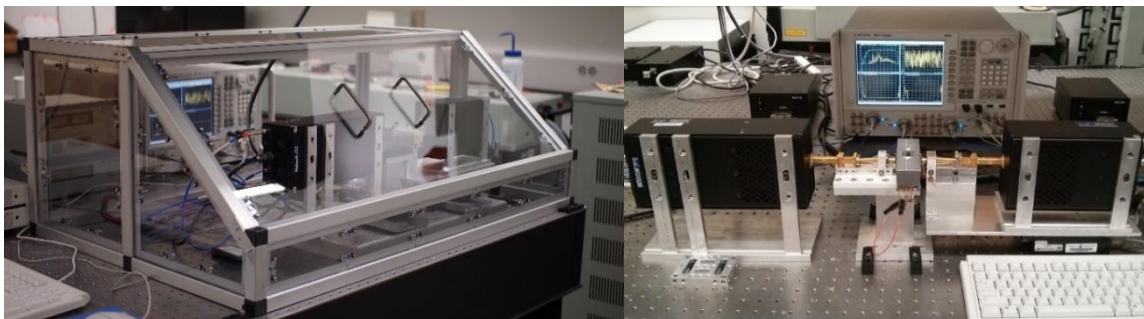


Figure 2.2: Terahertz frequency domain spectroscopy system for measuring dielectric response of aqueous solutions. Using this setup, we could measure the intensity change and phase shift of GHz-to-THz radiations through the samples.

In an effort to deepen our understanding of picosecond to microsecond dynamics of biomolecules and water molecules, we have made use of all these techniques to cover a wide range of frequencies from 50 MHz up to 1.12 THz. Thus, we have been able to generate a comprehensive image of molecular dynamics and motions as slow as rotations up to fast collective vibrations. Studying these dynamics was also beneficial in understanding hydration water properties. More detailed information about experimental and instrumental aspects in engineering designs and theoretical approaches in studying the physics of these dynamics are mentioned in following chapters for each study case.

Chapter 3

New terahertz dielectric spectroscopy for the study aqueous solutions

This chapter was adapted with only minor changes from the manuscript:

“Reproduced D. K. George, A. Charkhesht, and N. Q. Vinh, New terahertz dielectric spectroscopy for the study of aqueous solutions. Review of Scientific Instruments, 2015. 86(12): p. 123105., with the permission of AIP Publishing”

Some of the materials in abstract, sections 3.2 and 3.3 have, also, appeared in N. Q. Vinh’s (Thesis Advisor) conference proceeding:

N. Q. Vinh. "Probe conformational dynamics of proteins in aqueous solutions by terahertz spectroscopy." Terahertz Emitters, Receivers, and Applications VII. Vol. 9934. International Society for Optics and Photonics, 2016.

We present the development of a high precision, tunable far-infrared (terahertz) frequency-domain dielectric spectrometer for studying the dynamics of biomolecules in aqueous solutions in the gigahertz-to-terahertz frequency in this chapter. As an important benchmark system, we report on the measurements of the absorption and refractive index for liquid water in the frequency range from 5 GHz to 1.12 THz (0.17 to 37.36 cm^{-1} or 0.268 to 60 mm). The system provides a coherent radiation source with power up to 20 mW in the gigahertz-to-terahertz region. The dynamic range of our instrument reaches 10^{12} and the system achieves a spectral resolution of less than 100 Hz . The temperature of samples can be controlled precisely with error bars of $\pm 0.02\text{ }^{\circ}\text{C}$ from $0\text{ }^{\circ}\text{C}$ to $90\text{ }^{\circ}\text{C}$.

3.1. Introduction

Terahertz frequency radiation provides unique opportunities to probe the picosecond to nanosecond timescale dynamics properties of biomaterials in liquid water [29-31]. The dissolved biomolecules exhibit low-frequency collective vibrational modes corresponding to conformational changes of biomolecules, such as, for example, the twisting and deformation of the DNA double-helix structure that can be probed directly by terahertz radiation [32]. It has been suggested that these low-frequency modes in hydrated biomolecules efficiently direct reactions and energy transport in biological systems.

Nonetheless, detailed knowledge of the structure and dynamics of biomolecules in aqueous solutions remains to be an outstanding problem in the physical and biological sciences. Furthermore, in the basic case, our understanding of the translational and rotational diffusion of water molecules and larger-scale rearrangements of its hydrogen-bonding network appears to be incomplete as significant debates exist regarding the vibrational and relaxational responses of water molecules at the femtosecond to picosecond timescales [29, 31, 33-35]. Unlike infrared and Raman spectroscopies, which are sensitive to femtosecond-scale intramolecular dynamics (i.e., bond vibrations), spectroscopy in the terahertz regime is sensitive to picosecond intermolecular solvent dynamics (i.e., molecular rotations associated with hydrogen bond breaking) as well as internal motions of solvated biomolecules. Spectroscopy in this regime thus provides a new window to study the dynamics of hydrated biomolecules, bulk solvent, and the water in the hydration shells of dissolved biomolecules. Unfortunately, the extremely strong absorbance of water, technical limitations associated with this frequency range and often severe interference artifacts have reduced the precision of prior terahertz spectroscopy studies. These obstructions limit our ability to characterize the largest-scale, most strongly interacting dynamic modes.

On the optical side of the electromagnetic spectrum, a number of techniques have been reported for the absorption as well as refractive spectroscopy in the terahertz region. Fourier transform spectroscopy (FTS) or Michelson interferometry is a popular technique for broad frequency applications in the infrared to mid-infrared frequencies. This technique obtains information on both the refractive index and the absorption properties of the sample. The technique employs a broadband radiation source which can cover the far-infrared or the terahertz region. However, the power of a typical light source at terahertz frequencies is very weak, limiting the signal-to-noise of the technique in this region. Liquid water is highly absorbing in the terahertz frequencies, thus measurements have been done with a thin layer of water in the transmission [36, 37] or in the reflection [38, 39] configurations. In order to increase the signal-to-noise of the method at the terahertz region, measurements have been taken with far-infrared gas lasers containing methanol or methyl iodide at low pressures with powers of several mW [40, 41]. This method is limited to a number of discrete wavelengths depending on the gas (typically, a few laser wavelengths from 95 μm to 1258.3 μm) due to discrete rotational transitions.

Terahertz frequency radiation provides unique opportunities to probe the picosecond to nanosecond timescale dynamics properties of biomaterials in liquid water [29-31]. The dissolved biomolecules exhibit low-frequency collective vibrational modes corresponding to conformational changes of biomolecules, such as, for example, the twisting and deformation of the DNA double-helix structure that can be probed directly by the terahertz radiation [32]. It has been suggested that these low-frequency modes in hydrated biomolecules efficiently direct reactions and energy transport in biological systems. Nonetheless, detailed knowledge of the structure and dynamics of biomolecules

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Recently, the absorption of liquid water using Free-Electron Lasers [42], synchrotrons [43], and a germanium laser [44] with high radiation power at terahertz frequencies have been reported. However, the lasers provide only limited tunability over a short range of frequency and only the absorbance (not the refractive index) of the liquid water could be extracted from the measurements. In some previous studies on protein solutions [45, 46], attempts have been made to extract the protein absorption coefficient by directly comparing it with that of a blank buffer. These treatments assumed that the absorption of the solution is a weighted sum of the absorption of its constituents. This assumption is not physically justified. This is especially true when the refractive index changes rapidly with frequency as in the case of aqueous solutions in the terahertz frequencies [16, 35, 47-49].

In terahertz time-domain spectroscopy [49-53], typically a femtosecond laser pulse generates a fast current pulse (~ 1 ps) in a dipole antenna fabricated on low-temperature grown GaAs. This leads to the emission of electromagnetic pulse. The waveform is then Fourier transformed to obtain the power spectrum in the terahertz range from 200 GHz to several THz, depending on the material, the structure of the antenna, and the duration of the fs pulse. It is a fast method with good reproducibility and it yields information on the real and imaginary components (or the absorption and refractive index) of materials. The disadvantage is the steep power roll-off leading to low signal-to-noise ratio for higher frequencies in the terahertz region.

On the microwave side of the spectrum, dielectric spectroscopy has been employed to provide information of the microstructure and molecular dynamics of liquid systems, especially for aqueous solutions. Barthel J. *et al.* [54] and Kaatz *et al.* [55] used the

microwave waveguide interferometer in the transmission configuration and coaxial-line reflection probe to obtain the dielectric relaxation spectra of water up to 89 GHz. The techniques measure simultaneously the absorption and refractive index of solution samples in a broad frequency range but is limited to the GHz frequency. In summary the main problem in the terahertz spectroscopy is the lack of high power, high dynamics range, high resolution and a large tunable frequency of radiation sources that limit us to study the conformational dynamics of biomolecules in the nature environment.

Here we introduce our terahertz frequency-domain spectrometer, which combines the important elements of high dynamic range with high power, tunable frequency, broadband emission in a tabletop experiment, demonstrating accurate absorption and refractive index measurements of aqueous solutions. We demonstrate that the terahertz frequency-domain spectrometer is a powerful tool for the dielectric spectroscopy in the gigahertz-to-terahertz frequency. As a first fundamental test sample we have studied pure water. Water plays an active and complex role in sustaining life, without it cells would cease to function. A deeper understanding of water will shed light on the physics and functions of biological machinery and self-assembly. However, the experimental literature describing the dynamics of water is often contradictory [46, 56-59]. The large dynamic range of our system eliminates the severe restriction on sample thickness that is typical to most terahertz spectrometers and therefore minimizes problems associated with multiple reflections of the incident light (standing waves, etalon effect). We have measured the absorption and refractive index of water and aqueous solutions over the 3rd order of magnitude range from gigahertz to terahertz frequencies. The system closes the gap between microwave region and the mid-infrared which is well established by the FTIR technique.

3.2. Experimental Setup

In an effort to improve our understanding of the picosecond dynamics of water and solvated molecules, we have built a gigahertz-to-terahertz frequency-domain dielectric spectrometer that supports the simultaneous measurements of absorbance and refractive index of solutions over the spectral range from 5 GHz to 1.12 THz (0.17 cm^{-1} to 37.36 cm^{-1} mm or 0.268 to 60 mm). The signal-to-noise and spectral resolution of this device are significantly improved relative to any previous state-of-the-art instruments. For example, while the dynamic range of a commercial terahertz time-domain spectrometer is just 10^6 and its spectral resolution is several gigahertz, the dynamic range of our instrument reaches an unprecedented value of 10^{12} and the system achieves a spectral resolution of less than 100 Hz (Figure 3.1). The system provides a coherent radiation source with a power up to 20 mW in the gigahertz-to-terahertz region. With the high power, we are able to measure

thick layers up to 2 mm of liquid water. The temperature of liquid sample can be controlled with high accuracy of (± 0.02) °C. Given these attributes, our spectrometer provides unique capabilities for the accurate measurement of even aqueous solutions known as strong absorbing materials [16, 35].

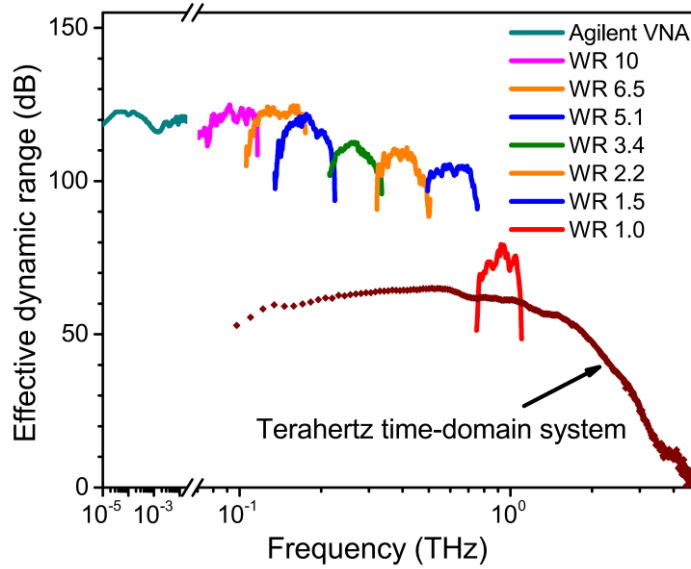


Figure 3.1: Dynamic range of our gigahertz-to-terahertz frequency-domain spectrometer (Agilent Vector Network Analyzer and frequency extenders from WR10 to WR1.0 systems) is compared with the dynamic range of a typical terahertz time-domain system. For WR10, WR5.1, WR6.5 and WR3.4 bands, we obtain the dynamic range measurements using a DUT with 30 dB loss [16].

Our spectrometer consists of a commercial Vector Network Analyzer (VNA) from Agilent, the N5225A PNA, which covers the frequency range from 10 MHz to 50 GHz, and frequency multipliers and the matched harmonic detectors for terahertz radiation, which are developed by Virginia Diodes, Inc. (Charlottesville, VA). Detailed information about the vector network analyzer frequency extension modules and the mixer process can be obtained elsewhere [60, 61]. The principle of the frequency extender terahertz modules is shown in Figure 3.2. Instead of using optical sources and mixing down the frequency to access the terahertz range, the terahertz radiation in this case is generated by up-converting frequencies from microwave sources. The frequency multipliers are fabricated using Schottky diode based components [61].

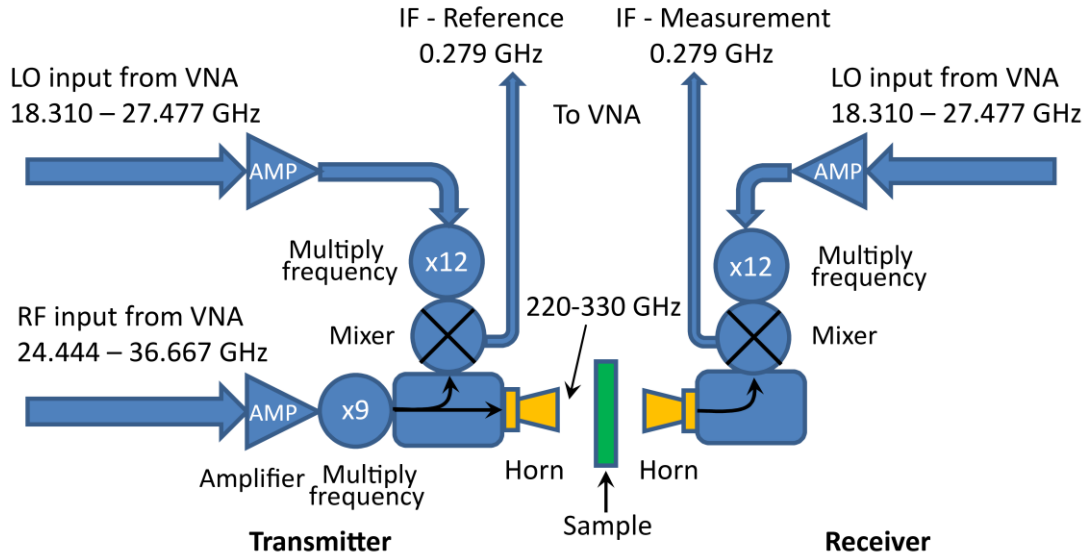


Figure 3.2: Block diagram of the WR3.4 (220 - 300 GHz) transmitter and receiver frequency extender modules. The microwave source from Agilent Vector Network Analyzer is extended via custom Virginia Diode frequency extenders to cover up to 1.12 THz [16].

The transmitter module allows to up-convert an arbitrary signal from a vector network analyzer in the frequency range between 10 MHz and 50 GHz to the terahertz frequency region and transmit it with a rectangular-to-circular horn antenna into free-space. Specifically, in Figure 3.2, the RF (radio frequency) input from a VNA with frequency range from 24.444 to 36.667 GHz enters the WR3.4 frequency extension modules for up conversion frequency by nine times to 220 to 330 GHz. At the receiver module a second horn antenna serves to receive the signal after a sample and feeds it into the mixer for down conversion. The transmitted as well as received signals mix with a Local Oscillator (LO) from the VNA in a subharmonic mixer. The resulting Intermediate Frequency (IF) signals from the transmitter and receiver detected by the VNA determine the intensity and phase of the reference and measurement signals. In this case, the IF signals are the difference between up-converted signals of RF and LO signals at 0.279 GHz. Our terahertz sources from Virginia Diodes for the WR3.4 frequency extension module produce several milliwatts of power at 300 GHz. The dynamic range for this frequency band of 110 dB can be achieved with a device under test (DUT) of 30 dB loss (Figure 3.1).

The spectrometer provides a large range of frequencies from gigahertz to terahertz with the output power up to 20 mW. The frequency extenders consist of commercial frequency extenders and matched harmonic receivers from Virginia Diodes, Inc. including WR10, WR6.5, WR5.1, WR3.4, WR2.2, WR1.5 and WR1.0 to cover the frequency range from 60 GHz to 1.12 THz. The dynamic range of the instrument reaches 1012 with a spectral resolution of less than 100 Hz (Figure 3.1). The lower frequency bands including

WR10, WR5.1, WR6.5 and WR3.4 have high output power up to 20 mW, thus we obtain the dynamic measurements for these bands using a DUT with 30 dB loss.

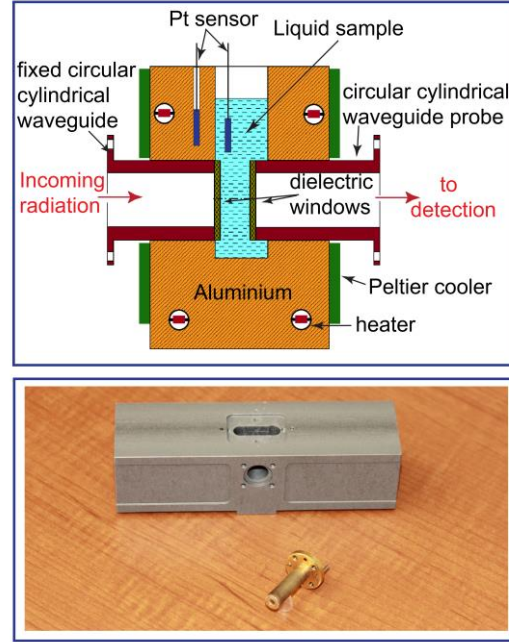


Figure 3.3: A variable path-length sample cell measures how absorbance and refractive index change with changing path-length of sample cell (top). The sample cell with the WR10 circular horn allows us to measure the dielectric response of liquid materials from 60 GHz to 1.12 THz (bottom) [16].

For convenience to change frequency bands, the output radiation from WR6.5, WR5.1, WR3.4, WR2.2, WR1.5 and WR1.0 frequency extenders is transformed into the rectangular WR10 waveguide configuration with waveguide taper transitions. From the rectangular WR10 waveguide, we use a transition waveguide to transform the radiation into the circular WR10 waveguide with minimum loss and reflections. The output of the circular WR10 horn enters our sample cell (Figure 3.3). The internal diameter of the circular horn is 2.85 mm and the wall thickness at the end of the horn is 2.00 mm. Thus we can easily obtain the dielectric response from 60 GHz to 1.12 THz for liquid samples. For lower frequencies from 5 GHz to 50 GHz, we employ directly the radiation from the VNA system into the sample cell designed for WR137 and WR28 waveguide configuration.

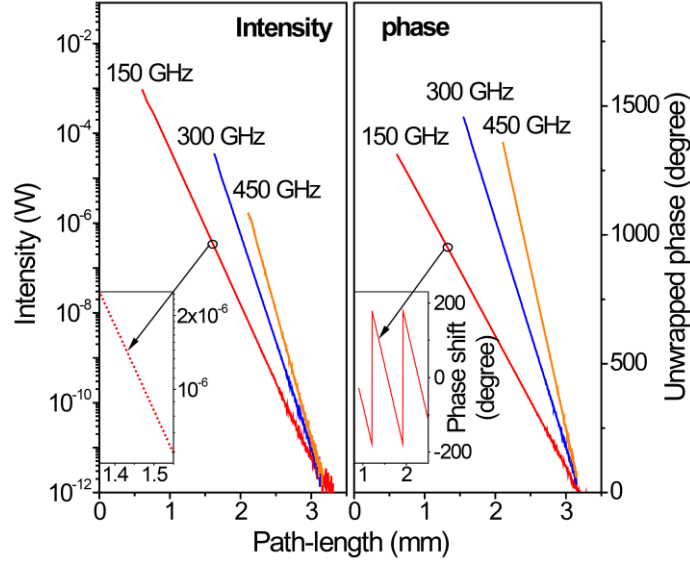


Figure 3.4: The variable path-length sample cell measures the intensity (left) and the phase (right) of transmitted terahertz radiation as functions of path-length. The slopes of these lines define the absorbance coefficient and refractive index of water, respectively, without the need for knowledge of the (difficult to obtain) absolute path-length or absolute absorbance of our samples. The insets to the figure demonstrate the quality of the measurements. The data in the right inset illustrate the phase shift as a function of sample length [16].

We have employed a variable path-length cell setup [42, 49] consisting of two parallel windows inside an aluminum cell, one immobile and the other mounted on an ultra-precise linear translation stage (relative accuracy of 50 nm) (Figure 3.3, top). Our translation stage from Newport (XMS160 ultra-precision linear Motor Stages) can perform 1 nm minimum incremental motion with a travel range of 160 mm. The linear translation stage has a direct-drive system for ultra-precision and a high accuracy linear glass scale encoder with 80 nm repeatability. We use thin polyethylene sheets of 80 μm thickness for the two parallel windows to cover the circular side of the horn antenna with the internal diameter of 2.85 mm. The large thickness of the wall at the end of the circular waveguide allows us to glue the windows strongly so that they retain their shape during measurements (Figure 3.3, bottom). The thin parallel windows avoid the multi-reflection effect to the radiation source as well as the detection part. The metal cell minimizes the leakage of stray radiation. The thickness of liquid samples or the distance between the two windows, which is the sample path-length, is adjusted using the ultra-precise linear stage. At each frequency, we examine an average of 100 different path-lengths (Figure 3.4), with increments ranging from 0.1 to 20 μm , depending on the absorption strength of the sample.

The choice of thickness of liquid water sample depends on the dynamics of frequency bands. The thickness varies from 0.5 mm for WR1.0 to 2.0 mm for WR10 band.

Since the system is frequency-domain, we can use the frequency step size as small as the linewidth of the radiation (sub 100 Hz). Depending on the spectral linewidth of the material, we will choose the frequency step size. Water in the gigahertz to terahertz frequencies shows a broad band of absorption and refractive index. Typically, we use a frequency step size of 1 GHz for water measurements.

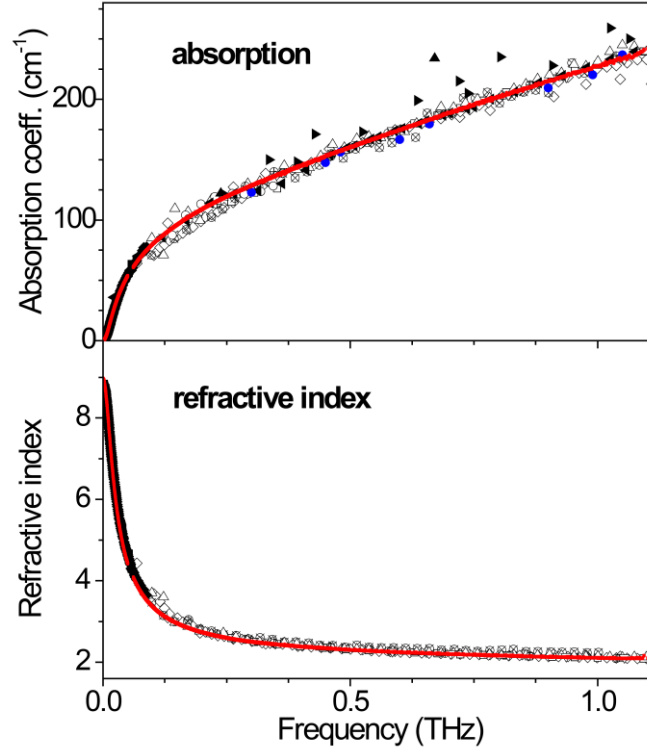


Figure 3.5: The red, continuous lines on these two plots are water spectra collected with our instrument. The error bars of absorption and refractive index measurements are within the thickness of the lines. Superimposed on these are data collected from the literature including measurements using FTIR interferometer (∇ [36] and $\blacktriangleleft$ [37]), reflection dispersive Fourier transform spectroscopy ($\oplus$ [38] and $\circ$ [39]), far-infrared lasers ($\blacktriangle$ [40] $\blacktriangleright$ [41]), free-electron laser ($\bullet$ [42], terahertz time-domain transmission ($\diamond$ [49, 50], $\square$ [51]) and reflection ($\otimes$ [52] $\triangle$ [53]) spectroscopies, dielectric relaxation spectroscopy ($\blacklozenge$ [54] $\blacksquare$ [55]) [16].

The fast performance and signal acquisition of the system of 35 μ s per frequency allow us to perform time dependent measurements. We employ the high speed ethernet connection for data acquisition to transfer data from the VNA to a computer. The time to obtain both absorption and refractive index measurements for one frequency extender system varies from 20 seconds to 5 minutes depending on the number of frequency points. For example, to scan from 5 GHz to 1.12 THz with an average of five times and a frequency step size of 1 GHz for water measurements (Figure 3.5) requires about 3 hours including measurement time and 5 minutes each time to change frequency extenders.

The temperature of the sample cell can be controlled precisely from 0 °C to 90 °C. The sample cell is embedded in a large metal body part of 152 x 38 x 18 mm (Figure 3.3). The Peltier cooler plates from Custom Thermoelectric (12711-5L31-03CK) and high power resistors are mounted on the body of the sample cell, allowing precise control of the temperature of the sample. The absorbance and refractive index of water are extremely sensitive to temperature, and thus all experiments are carried out with a measured accuracy of ± 0.02 °C. To mitigate problems associated with multiple reflections of the incident light (standing waves, etalon effect), the thickness of our shortest path-length was selected to be long enough to ensure strong attenuation of the incident radiation (transmission $<10^{-2}$).

3.3. Data Evaluation

3.3.1. Absorption and refractive index measurements:

Using the above-described spectrometer and sample cell, we have measured the change of intensity and phase in aqueous samples as functions of path-length (Figure 3.4). The absorption process of the terahertz radiation passing through a sample is described by Beer's law:

$$I(l, \nu) = I_0(\nu) \cdot e^{-\alpha(\nu) \cdot l} \quad (3.1)$$

where ν , I_0 , I , $\alpha(\nu)$ and l are the frequency, the incident intensity, the intensity at the detection of the radiation, the absorption coefficient as a function of radiation frequency, and the thickness of the sample, respectively. When the radiation passes through a material, it will always be attenuated. This can be conveniently taken into account by defining a complex refractive index:

$$n^*(\nu) = n(\nu) - i\kappa(\nu) \quad (3.2)$$

where the real part, $n(\nu)$, is the refractive index and indicates the phase velocity, while the imaginary part, $\kappa(\nu)$, is called the extinction coefficient and indicates the amount of attenuation when the radiation propagates through the material. The extinction coefficient is associated with the absorption coefficient, $\alpha(\nu)$, by

$$\alpha(\nu) = \frac{4\pi \cdot \nu \cdot \kappa(\nu)}{c} \quad (3.3)$$

with c is the speed of light. We have measured the intensity and phase shift of water and aqueous solutions over the three order of magnitude range 5.0 GHz – 1.12 THz as functions of path-length, l , at 20.00 (± 0.02) °C. The absorption coefficient is determined by the slope

of a linear fit of $\ln I(l, \nu)$ to the path-length, l , without the need for precise knowledge of the sample's absolute absorbance or absolute path-length:

$$\ln I(l, \nu) = \ln I_0(\nu) - \alpha(\nu) \cdot l. \quad (3.4)$$

Alternatively, the refractive index, $n(\nu)$, can be calculated by fitting the measured phase shift $\theta(l, \nu)$ as a linear function of path-length of the sample:

$$\theta(l, \nu) = \theta_0(\nu) + \frac{2\pi \cdot \nu \cdot n(\nu)}{c} \cdot l \quad (3.5)$$

where $\theta_0(\nu)$ is the phase of the reference signal. Note that eq. (3.5) does not have a form of $(n(\nu) - 1)$ as in many variable path-length measurements since our detector is attached to the moving window of the sample cell. When the optical path-length of the sample is changed, the detector moves with a distance that is equivalent to the change of the geometrical length of the light traveling in the sample. Both properties of liquid water (absorption coefficient and refractive index) as functions of frequency, are growing and falling, respectively, with increasing frequency over this entire spectral range (Figure 3.5).

This method supports the precise determination of absorption coefficients and refractive indexes without the need for precise (and difficult to obtain) measurements of the absolute path-length and the intrinsic optical properties of the sample cell. All experiments were repeated approximately five times to estimate confidence limits. We fit the intensity data to the Beer's law, eq. (3.1), to obtain values for the absorption coefficients of a sample as a function of frequency, α (Figure 3.4, left) with a high degree of accuracy. In parallel, fitting the measured phase shift as a linear function of path-length, eq. (3.5), provides the refractive index of the sample, n (Figure 3.4, right). The standard errors of the mean of replicate measurements are typically smaller than 0.2%. Using our sensitive setup, we measured precisely the absorption coefficient and refractive index of the strong absorption material, water (Figure 3.5), and aqueous solutions at the terahertz frequencies. The red, continuous lines on these two plots in Figure 3.5, are water spectra collected with our instrument at 20 °C. The error bars of our absorption and refractive index measurements are within the thickness of the lines. Superimposed on these are data collected from the literature [38, 39, 42, 49, 52, 62, 63] illustrating the vastly enhanced spectral resolution and the signal-to-noise and of our instrument.

3.3.2. Complex dielectric response of solutions:

The spectroscopies cover a broadband spectral range from gigahertz to terahertz frequencies that allow us to observe both the relaxational (rotational) and translational

processes of waters and biomolecules. Thus, the dielectric response will provide an entire picture of the dynamics of biomolecules in the living environment. The frequency-dependent complex dielectric response, $\varepsilon^*(\nu) = \varepsilon'(\nu) - i\varepsilon''(\nu)$, is related to the complex refractive index, $n^*(\nu) = n(\nu) - i\kappa(\nu)$, through the relations [64]:

$$\begin{aligned}\varepsilon'(\nu) &= n^2(\nu) - \kappa^2(\nu) = n^2(\nu) - (c\alpha(\nu)/(4\pi\nu))^2, \\ \varepsilon''(\nu) &= 2n(\nu) \cdot \kappa(\nu) = 2n(\nu)c\alpha(\nu)/(4\pi\nu).\end{aligned}\tag{3.6}$$

From this we have been able to determine the complex dielectric function, $\varepsilon^*(\nu)$, of the water and aqueous solutions, which sequentially offers a complete explanation of the interaction of the solution with the incoming electromagnetic wave. Figure 3.6 shows the dielectric response from water at 20 °C. Conversely, given the complex dielectric response, $\varepsilon'(\nu)$ and $\varepsilon''(\nu)$, we can determine the absorption, $\alpha(\nu)$, and the refractive index $n(\nu)$:

$$\begin{aligned}n(\nu) &= \left(\frac{\sqrt{\varepsilon'(\nu)^2 + \varepsilon''(\nu)^2} + \varepsilon'(\nu)}{2} \right)^{1/2}, \\ \alpha(\nu) &= \frac{4\pi\nu}{c} \left(\frac{\sqrt{\varepsilon'(\nu)^2 + \varepsilon''(\nu)^2} - \varepsilon'(\nu)}{2} \right)^{1/2}.\end{aligned}\tag{3.7}$$

3.4. Discussion

We have demonstrated that we are now able to determine the absorption as well as the refractive index of aqueous solutions with the dielectric terahertz spectroscopy. The system reduces the influence of interference and other systematic effects to minimum and provides reliable absolute experimental data for water and aqueous solutions. The absorption coefficient as well as the refractive index of water strongly depend on temperature. We have employed the Peltier system to control the temperature with a high accuracy of 0.02 °C. It is a fast technique that allows us to observe the conformation changes of biomolecules in solutions as a function of time.

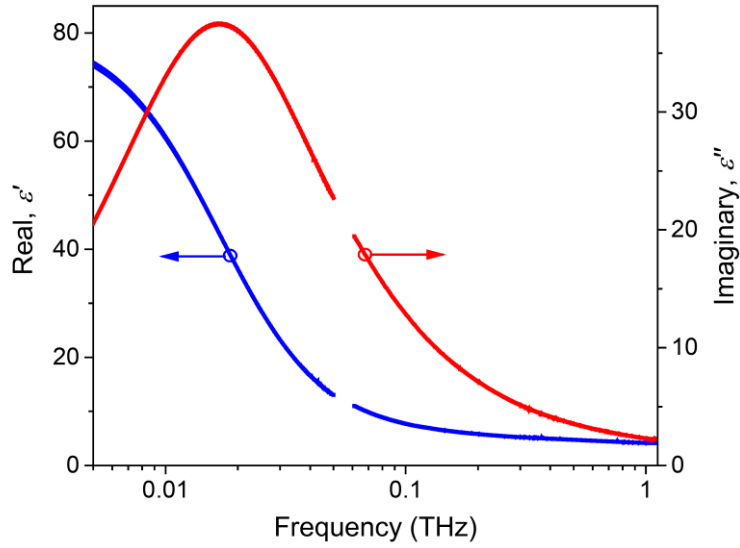


Figure 3.6 The dielectric response from water at 20 °C is converted from the absorption coefficient and refractive index measurements. The error bars for the calculated dielectric response are within the thickness of the lines [16].

Figure 3.5 shows the result of our measurement of liquid water measured at 20 °C. The absorption coefficient, and refractive index are strong functions of frequency, monotonically increasing and decreasing, respectively, with rising frequency over this entire spectral range. The red, continuous lines collected with our instrument for water spectra are in good agreement with previously reported spectra (Figure 3.5). Zelsmann [36] and Zoidis *et al.* [37] recorded the water spectra with a FTIR interferometer in the range from 8 to 450 cm^{-1} (239 GHz to 13.5 THz) using the transmission configuration. Afsar *et al.* [38] and Hasted *et al.* [39] employed a reflection dispersive Fourier transform spectroscopy to measure to absorption and refractive index of liquid water in the spectral range between 4 and 450 cm^{-1} (120 GHz to 13.5 THz). Using far-infrared gas lasers with powers of several mW, Vij *et al.* [40] and Simpson *et al.* [41] reported the absorption coefficients of liquid water in a few frequencies in the terahertz region. Xu *et al.* [42] measured the absorption coefficient of liquid water between 0.3 to 3.75 THz with free-electron lasers. Schmuttenmaer *et al.* [49, 50] and Yada *et al.* [51] reported the absorption coefficient and refractive index with a terahertz time-domain system in the transmission configuration in the range from 2.0 to 60 cm^{-1} (60 GHz to 1.8 THz) using a variable path-length sample cell. The absorption coefficient was determined by the slope of a linear regression fit of the detected intensity versus the path-length at room temperature. Thrane, Ronne *et al.* [52, 53] collected the terahertz spectrum of liquid water at 292 K using a terahertz time-domain system in the reflection configuration in the range from 3.0 to 33 cm^{-1} (90 GHz to 1.0 THz). Barthel J. *et al.* [54, 56] and Kaatz *et al.* [55] used the microwave waveguide interferometer in the transmitted configuration and coaxial-line

reflection probe, reflectively, to obtain the dielectric relaxation spectra of water up to 89 GHz. Superimposed on these are data collected from the literature, [38, 39, 41, 42, 49, 52, 62, 63] illustrating the significantly improved signal-to-noise and spectral resolution of our terahertz spectrometer.

In summary, we have demonstrated a new method to obtain terahertz spectra of highly absorption polar liquid with a high precision, high dynamics range, high resolution and a large frequency range from gigahertz-to-terahertz region. The terahertz frequency-domain dielectric spectroscopy applied to liquid water obtained a good agreement with previous measurements. Using this setup, we have been able to determine the absorption coefficient and the refractive index of water as well as the aqueous biological solutions in the range between 5 GHz and 1.12 THz with high precision.

Chapter 4

High-Precision Megahertz-to-Terahertz Dielectric Spectroscopy of BSA Protein Collective Motions and Hydration Dynamics

This chapter was adapted with only minor changes from the manuscript:

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The low-frequency collective vibrational modes in proteins as well as the protein-water interface have been suggested as dominant factors controlling the efficiency of biochemical reactions and biological energy transport. It is thus crucial to uncover the mystery of hydration structure and dynamics as well as their coupling to collective motions of proteins in aqueous solutions. Here we report dielectric properties of aqueous BSA protein solutions as a model system using an extremely sensitive dielectric spectrometer with frequencies spanning from megahertz to terahertz. The dielectric relaxation spectra reveal several polarization mechanisms at the molecular level with different time constants and dielectric strengths, reflecting the complexity of protein-water interactions. Combining the effective-medium approximation and molecular dynamics simulations, we have determined collective vibrational modes at terahertz frequencies and the number of water molecules in the tightly-bound and loosely-bound hydration layers. High-precision measurements of the number of hydration water molecules indicate that the dynamical influence of proteins extends beyond the first solvation layer, to around 7 Å distance from the protein surface, with the largest slowdown arising from water molecules directly hydrogen-bonded to the protein. Our results reveal critical information of protein dynamics and protein-water interfaces, which determine biochemical functions and reactivity of proteins.

4.1. Introduction

Biological functions of proteins in aqueous environments, such as enzymatic activity, oxygen transport, neuron signal transmission, and ion channels for signaling currents, depend on their structural changes, flexibility, and protein-water interface [32, 65-70]. Specifically, protein flexibility and vibrational modes have been considered to be responsible for efficiently directing biochemical reactions and biological energy transport.

It has been suggested that low-frequency collective vibrational modes (<3 THz) involving dynamical networks extending throughout the protein play a crucial role in controlling the structural changes. As a general rule, biological functions of proteins occur in aqueous environments [71]. While solvation effects on proteins play an essential role in the structure, stability, and dynamics of proteins, our understanding of solvent dynamics at the protein-water interface remains inadequate. Water molecules that populate the surfaces of proteins [35, 72, 73], lipid bilayers [74], lipid headgroups [75], or in the crowded milieu of tissues and cells [30] exhibit properties that are particularly important to the structure and biological functions of a protein, and distinct from those found in the bulk. With a small size and a large dipole moment, water molecules form stable layers around proteins to perform a multitude of functions in biological environments. The structure and dynamics of these layers, which are determined by hydrophobic and hydrophilic interactions, among other influences, are important for biological functions. Our understanding of the flexibility of a protein and how its environment contributes to catalytic mechanisms lags far behind our knowledge of three-dimensional structures and chemical mechanisms.

A wide range of experimental and computational techniques have been employed to investigate the molecular dynamics of proteins in solution [76]. Different techniques may provide information on different aspects of protein dynamics. For example, some techniques, such as nuclear magnetic resonance (NMR) [69, 77] and Mössbauer spectroscopy [78, 79], are used to detect the motion of probe nuclei, while others including optical pumping [80, 81], femto-second pump-probe [82-84], optical Kerr-effect [73, 85], and inelastic neutron scattering experiments [86, 87] report on the dynamics of more globally distributed probes. Some experiments (e.g., inelastic neutron scattering, NMR) require complex facility-based methods as well as cryogenic temperatures and/or non-physiological conditions. The measured timescales of protein motions also differ between techniques [65]. X-ray crystallography collects average atomic positions over hours; Mössbauer spectroscopy probes motions on a timescale of about 10^{-7} s; neutron scattering detects motions on timescales ranging from 10^{-12} to 10^{-8} s, depending on instrumental resolution. Molecular dynamics (MD) simulations [88-91] are largely limited by accessible timescales which at best can reach 10^{-6} s (though some recent MD simulations of protein dynamics can reach 10^{-3} s timescale but require special hardware). One approach, megahertz (MHz) to terahertz (THz) dielectric spectroscopy, provides observations that are relevant to the global and subglobal motions on the picosecond to sub-microsecond timescales [35, 75, 92, 93]. The dielectric spectroscopy is a label-free technique that is carried out at physiological conditions of proteins. However, far-infrared and terahertz time-domain spectroscopy (THz-TDS) have been limited by the very large absorption of liquid water. Although recent THz-TDS experiments on lysozyme crystals have successfully identified underdamped delocalized vibrational motions in the terahertz frequencies, the protein dynamics are strongly affected by crystal packing [32].

The properties of hydration water such as dielectric susceptibility and relaxation frequency are strongly affected by the slow but large-scale motion of a protein molecule [35, 75, 94]. However, using THz radiation, several groups reported different results for the effects of proteins on water structure. For example, Ding et al. [95] estimated a hydration layer with a thickness of $\sim 11\text{--}17$ Å from the peptide surface using THz-TDS experiments. They argued that effects of a peptide surface in an aqueous solution are beyond the first hydration shell, but no long-range effect on water structure has been identified. Employing a p-germanium laser at 2.25 and 2.55 THz, Ebbinghaus et al. [45, 96] reported a nonlinear behavior of THz absorption with protein concentration, which was attributed to the onset of overlapping dynamical hydration layers, leading to conclusions that a protein has a long-range effect on water beyond 20 Å from the protein surface. The suggestion became controversial as it was in contrast with previous findings of MD simulations [88], protein functional studies [97], magnetic relaxation dispersion measurements [98], and high precision densitometry experiments [99]. These aforementioned works concluded that proteins dominate only one hydration layer rather than multiple hydration layers. Recently, on the basis of “hydration layer overlap” hypothesis, Bye et al. [100] used a modified calculation model to explain the variation in absorption coefficients with protein concentration. However, this model does not take the absorption of proteins in the THz frequencies into consideration, which makes the explanation lose its universality. The controversy may also originate from distinct definitions of hydration layers, and the sensitivity of the apparatus. Given the controversy resulting from these works, it is important to investigate the validity of the explanation of the measurements.

Advances in MHz-to-THz technology make it possible to conduct a more thorough study of the dielectric response of proteins in an aqueous environment. Such a study can act as an important step in understanding motions of complex biomolecular systems at timescales from microsecond to picosecond. Here, we report the dielectric response from MHz-to-THz spectroscopy of a benchmark protein, bovine serum albumin (BSA), in aqueous solutions. Through our developments we have achieved a very large spectral bandwidth from MHz to THz frequencies and improved the signal-to-noise ratio by several orders of magnitude, providing continuous high-fidelity coverage that no other techniques can match [16]. Employing high-precision measurement, we systematically study the nature of biological water and the associated dynamics of proteins at the molecular scale.

4.2. Experimental Methods

4.2.1. Sample Preparation

BSA proteins with a molecular weight of 66.1 kDa purchased from Sigma Aldrich (Cat. No. 9048-46-8) were used to prepare BSA solutions. In order to accurately determine

the molar concentration as well as the volume filling factor of the protein, f_p , BSA proteins were dissolved in 5 ml of deionized water in a volumetric flask. The solutions were prepared by weighing and measuring the volume after dissolving BSA in water for several times to obtain an accurate value of the partial specific volume. The accuracy of these measurements is 0.04 ml or 0.8 %. The temperature of BSA solutions was kept in the range above 0 - 5 °C in an ice box. Before the measurement, solutions were placed outside the ice-box to reach room temperature. We have determined accurately the dielectric response of BSA solutions from 100 MHz to 2.0 THz with concentrations ranging from μM to mM using a MHz-to-THz frequency-domain spectrometer based on a vector network analyzer [16, 35] and a THz time-domain spectrometer [101].

4.2.2. Dielectric Spectroscopy

Our spectrometers cover a broadband spectral range from MHz to THz frequencies that allows us to observe both the relaxational (rotational) and translational processes of water molecules (bulk, tightly- and loosely-bound water) as well as biomolecules. Figure 4.1a shows the absorption and refractive index of BSA solutions for several solutions. With simultaneous measurements of the absorption and refractive index, the complex refractive index, $n^*(\nu)$, of a material can be expressed as a function of frequency, ν :

$$n^*(\nu) = n(\nu) + i\kappa(\nu) \quad (4.1)$$

where $n(\nu)$ is the refractive index of the solution, and $\kappa(\nu)$ is the extinction coefficient of the solution and is related to the absorption coefficient, $\alpha(\nu)$, by $\kappa(\nu) = c\alpha(\nu)/(4\pi\nu)$ with c being the speed of light. Similarly, the complex dielectric response of a material can be expressed as:

$$\epsilon_{\text{sol}}^*(\nu) = \epsilon'_{\text{sol}}(\nu) + i\eta''_{\text{sol}}(\nu) \quad (4.2)$$

where $\epsilon'_{\text{sol}}(\nu)$ and $\eta''_{\text{sol}}(\nu)$ are the real (dielectric dispersion) and imaginary (total dielectric loss) components of the relative permittivity, respectively. The imaginary component of the dielectric response of BSA solutions contains two contributions:

$$\eta''_{\text{sol}}(\nu) = \epsilon''_{\text{sol}}(\nu) + \eta''_{\sigma}(\nu) = \epsilon''_{\text{sol}}(\nu) + \sigma/(2\pi\nu\epsilon_0) \quad (4.3)$$

where $\epsilon''_{\text{sol}}(\nu)$ represents the dielectric loss, $\eta''_{\sigma}(\nu)$ is the Ohmic loss due to the drift of ions contained in BSA solutions, σ is the electrical conductivity of the solution, and ϵ_0 is the permittivity of the vacuum. The electrical conductivity of BSA solutions has been obtained at the frequency of 1 kHz and at 25 °C. These values have been used for the extraction of the dielectric loss of BSA solutions from the total dielectric loss. Since we simultaneously

measure both the absorption and refractive index of materials, the complex dielectric response of BSA solutions can be calculated from the following relations:

$$\begin{aligned}\epsilon'_{\text{sol}}(\nu) &= n^2(\nu) - \kappa^2(\nu) = n^2(\nu) - (c\alpha(\nu)/4\pi\nu)^2, \\ \epsilon''_{\text{sol}}(\nu) &= 2n(\nu) \cdot \kappa(\nu) = \frac{2n(\nu)c\alpha(\nu)}{4\pi\nu}.\end{aligned}\quad (4.4)$$

The complex dielectric response spectra (dielectric dispersion, $\epsilon'(\nu)$, and the dielectric loss, $\epsilon''(\nu)$, components) of water and BSA solutions at 25 °C have been extracted from the absorption and refractive index measurements (Figure 4.1b). A main dielectric loss peak frequency centered at ~19 GHz remains virtually unchanged. This dielectric loss peak has been attributed to bulk water in the solution [16, 75, 102].

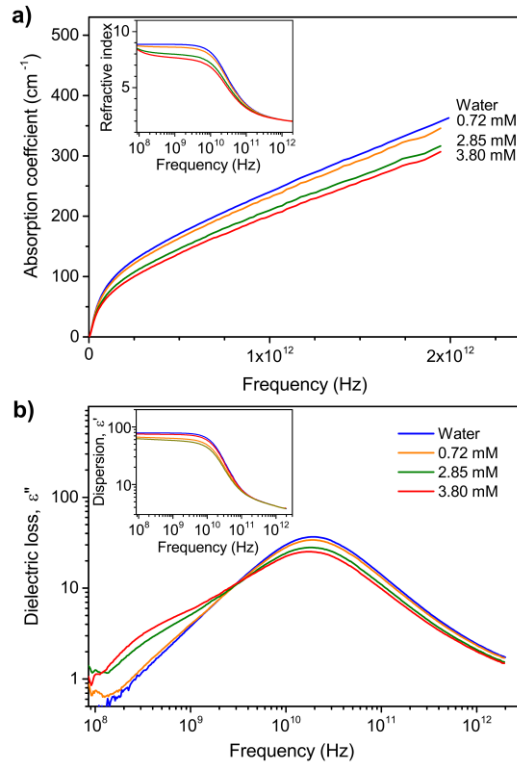


Figure 4.1: The interaction of MHz to THz radiation and BSA proteins providing the dynamics over picosecond to sub-microsecond timescales. **(a)** The MHz to GHz absorption of both BSA solutions and water rises monotonically with increasing frequency at 25 °C. The refractive indices (upper inset) of BSA solutions and water diminish with increasing frequency. **(b)** The dielectric loss, $\epsilon''(\nu)$ and the dielectric dispersion spectra, $\epsilon'(\nu)$, in the lower inset, from BSA solutions and water were obtained from the absorption and refractive index measurements. The main dielectric loss peak frequency centered at ~19 GHz remains unchanged. An addition of BSA proteins in solutions produces a pronounced broadening on the lower frequency side of the dielectric loss spectra [103].

However, the presence of BSA proteins in solutions induces a pronounced broadening of dielectric loss spectra on the lower frequency side of the main dielectric loss peak. The dielectric response for the orientational relaxation of molecular dipoles with the size of 66 kDa (BSA protein) is in the range of 1 to 10 MHz [75, 102]. Thus, the low-frequency broadening is not likely due to relaxation processes of bulk water as well as BSA proteins in the solution, but can be attributed to the emergence of new slow relaxation modes.

4.3. Results and Discussion

4.3.1. Megahertz to Gigahertz

Dielectric relaxation spectrometry of aqueous BSA solutions at MHz to GHz frequencies provides insight into mechanisms of reorientational dynamics of water. The technique is definitive for inferring the distinctly different dynamics of water molecules in hydration shells and those in the bulk (Figure 4.1).

At the microscopic level, several dielectric mechanisms or polarization effects contribute to the dielectric response of aqueous solutions. Water molecules as well as biomolecules with permanent dipoles rotate to follow an alternating electric field. In this frequency range, three contributions dominate in the dielectric response of aqueous protein solutions, including (i) the rotational motion of biomolecules, i.e., orientational relaxation of biomolecular dipoles; (ii) the orientational polarization of bulk water molecules, i.e., water dipoles in the bulk; (iii) the orientational polarization of water molecules in the interfacial region surrounding biomolecules, i.e., water dipoles in hydration shells. Atomic and electronic polarizations are relatively weak, and are usually constant over the MHz to GHz frequency range.

Dielectric spectra of BSA solutions in the low frequency region (below 50 GHz) are shown in Figure 4.2. Typically, the dielectric response of the orientational relaxation of permanent dipoles from macromolecules with a molecular weight of ~66 kDa is in the range of 1 to 10 MHz [75, 102]. We do not focus on this frequency range in this chapter. The dielectric response of the orientational polarization of bulk water centered at ~19 GHz has been well established [16, 59, 104]. However, the contribution of the dielectric response of water in hydration layers of proteins is complex and less understood. For example, the dielectric response of hydration water of ribonuclease A [105], lysozyme protein [102], and zwitterionic dodecylphosphocholine micelles [75] in aqueous solutions consists of several dispersion regions. Typically, in a simple approximation, hydration water can be classified into two types including tightly-bound water (with dielectric

response in 100 – 500 MHz) and loosely-bound water (with dielectric response in 1 – 5 GHz). The tightly-bound water molecules have direct and strong contacts with biomolecular surfaces. We have shown that water molecules having direct but weak interaction with biomolecular surfaces (such as with a soft cation) can be categorized as loosely-bound water [75]. Thus, loosely-bound hydration water include water molecules in outer hydration shells and water molecules having weak interactions with biomolecular surfaces, regardless of location. These loosely-bound water molecules exchange with tightly-bound water and have dynamics approaching those of bulk water.

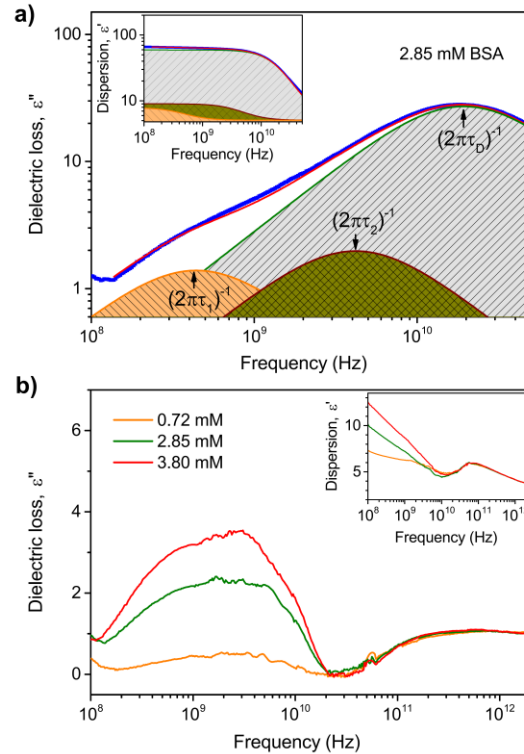


Figure 4.2: The dielectric response of BSA aqueous solutions in the frequency range from 100 MHz to 50 GHz showing the heterogeneity on a scale of several water layers around proteins. **(a)** Dielectric spectra for both dielectric dispersion (upper inset), $\epsilon'_{\text{sol}}(\nu)$, and dielectric loss, $\epsilon''_{\text{sol}}(\nu)$, together with their spectral deconvolution provide insight into the dynamics of water molecules at the protein surface for the 2.85 mM BSA solution. The red curves are fits of the real and imaginary components of the complex dielectric response. **(b)** The dielectric loss and dielectric dispersion (lower inset) spectra of tightly- and loosely-bound water for several BSA solutions have been obtained by subtracting the well-defined relaxation contribution of bulk water from the total spectra. The procedure reveals the distinctly different dynamic behavior of hydration layers compared to bulk water [103].

In the MHz to GHz frequency region, the data were analyzed by simultaneously fitting dielectric dispersion and dielectric loss components to relaxation models based on the sum of n individual contributions described by the Havriliak-Negami function [106]. The librational motions and inertial effects do not contribute appreciably to the dielectric response, yielding a usually constant (ϵ_∞) value over the region. Thus, the dielectric response is in the form:

$$\epsilon^*(\nu) = \epsilon_\infty + \sum_{j=1}^n \frac{\epsilon_{j-1} - \epsilon_j}{(1 + (i2\pi\nu\tau_j)^{1-\alpha_j})^{\beta_j}}. \quad (4.5)$$

Each mode is characterized by an amplitude (relaxation strength), $\Delta\epsilon_j = \epsilon_j - \epsilon_{j+1}$, a relaxation time, τ_j ($\tau_j > \tau_{j+1}$), and shape parameters, $0 \leq \alpha_j < 1$ and $0 < \beta_j \leq 1$. The simplified variants include the Cole-Davidson ($\alpha_j = 0; 0 < \beta_j \leq 1$), the Cole-Cole ($0 < \alpha_j \leq 1; \beta_j = 1$), and the Debye ($\alpha_j = 0; \beta_j = 1$) equations. In equation (4.5), $\epsilon_0 = \epsilon_s$ is the static permittivity which can be defined as $\epsilon_s = \epsilon_\infty + \sum_{j=1}^n \Delta\epsilon_j$, and $\epsilon_n = \epsilon_\infty$ is the dielectric constant at infinity frequency that captures contributions of dielectric response from modes with frequencies much higher than the probed range, and thus reflects contributions from atomic and electronic polarizations. We have fitted equation (4.5) simultaneously to the measured dielectric dispersion, $\epsilon'_{\text{sol}}(\nu)$, and the dielectric loss, $\epsilon''_{\text{sol}}(\nu)$, to minimize errors. Our analyses show that it is sufficient to consider Debye-type relaxations. Specifically, our data are reasonably fitted with a superposition of three Debye relaxation processes in the form:

$$\epsilon^*_{\text{sol}}(\nu) = \epsilon_\infty + \frac{\epsilon_s - \epsilon_1}{1 + i2\pi\nu\tau_1} + \frac{\epsilon_1 - \epsilon_2}{1 + i2\pi\nu\tau_2} + \frac{\epsilon_2 - \epsilon_D}{1 + i2\pi\nu\tau_D} \quad (4.6)$$

where $\Delta\epsilon_1 = \epsilon_s - \epsilon_1$, $\Delta\epsilon_2 = \epsilon_1 - \epsilon_2$ and $\Delta\epsilon_D = \epsilon_2 - \epsilon_\infty$ are dielectric strengths of each Debye process to the total relaxation from tightly-bound, loosely-bound, and bulk water, respectively, with τ_1 , τ_2 , and τ_D as the corresponding relaxation times. It is important to note that, at 25 °C, the dielectric spectra of pure water could be formally fitted by a sum of three Debye processes [16, 59], yielding $\tau_D \sim 8.27$ ps (19 GHz), $\tau_{D2} \sim 1.1$ ps (145 GHz), and $\tau_{D3} \sim 0.178$ ps (895 GHz). In the frequency range up to 50 GHz, the contributions of the dielectric response of bulk water from τ_{D2} and τ_{D3} modes are small and included in the ϵ_∞ parameter.

Using this approach, we fit both the dielectric dispersion, $\epsilon'_{\text{sol}}(\nu)$, and loss, $\epsilon''_{\text{sol}}(\nu)$. Six parameters in equation (4.6) were varied simultaneously, while the relaxation time for bulk water, $\tau_D \sim 8.27$ ps, was held fixed at the literature value [16, 56, 59, 107]. Dielectric spectra for both dielectric dispersion, $\epsilon'_{\text{sol}}(\nu)$, and dielectric loss, $\epsilon''_{\text{sol}}(\nu)$, together with their spectral deconvolution are shown in Figure 4.2a for a 2.85 mM BSA solution. The dielectric loss spectrum indicates three relaxation processes centered at 441 ± 23 MHz

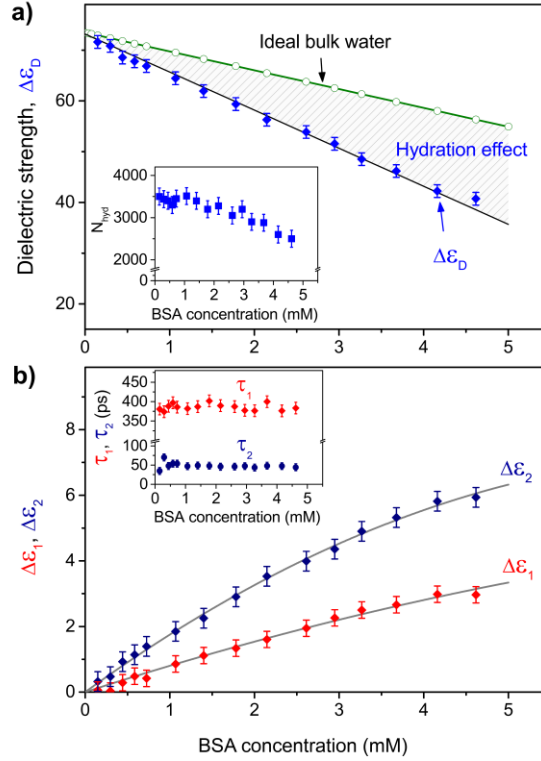


Figure 4.3: Dielectric relaxation measurements showing the existence of several relaxation processes in protein solutions. **(a)** The dielectric strength of the bulk water, $\Delta\epsilon_D$, in BSA solutions significantly decreases with increasing protein concentration. The continuous solid line (green) represents the dielectric amplitude of ideal bulk water calculated under an assumption that all water molecules in solution behave as bulk water and participate in the relaxation process at ~ 19 GHz. The hydration number, N_{hyd} , as a function of protein concentration (upper inset) deduced from the dielectric strength provides the number of water molecules that do not participate in the relaxation process of bulk water because of the hydration effect. **(b)** Amplitudes of the dielectric response of the tightly- and loosely-bound water in solutions increase with increasing protein concentration. The relaxation time constants (lower inset) of water in hydration shells are constant with protein concentrations [103].

($\tau_1 \sim 361 \pm 19$ ps), 4.19 ± 0.85 GHz ($\tau_2 \sim 38 \pm 11$ ps), and 19.25 ± 0.78 GHz ($\tau_D \sim 8.27 \pm 0.35$ ps) for tightly-bound, loosely-bound, and bulk water, respectively. Corresponding to the three relaxation processes in the dielectric loss spectrum, the dielectric dispersion is shown in the inset of Figure 4.2a. Our fitting to the three-Debye relaxational model in the low frequency region produces $\epsilon_\infty = 5.2 \pm 0.5$ for the 2.85 mM BSA solution. This value is within experimental uncertainty of the prior literature values [59, 107]. We have also obtained the values of the dielectric strength, with $\Delta\epsilon_1 = 2.8 \pm 0.3$ for tightly-bound, $\Delta\epsilon_2 = 3.9 \pm 0.3$ for loosely-bound, and $\Delta\epsilon_D = 54.3 \pm 0.3$ for bulk water, respectively. The dominant contribution from the τ_D relaxation process reflects

the cooperative reorientational dynamics of dipole moments of bulk water in the protein solution.

The dielectric measurements provide insights into the dynamics of water at the protein interface and the heterogeneous nature of hydration shells at a molecular level. Amplitudes of relaxation processes and relaxation time constants deduced from fitting experimental spectra to the three-Debye model (eq. (4.6)) are shown in Figure 4.3. Specifically, the relaxation times are independent of the protein concentration, c , over the entire concentration range that we have explored (Figure 4.3b, inset). Two long relaxation time constants, 379 ± 22 ps and 45 ± 11 ps, for the reorientation of water molecules in hydration shells have been identified, in addition to the relaxation time of bulk water of 8.27 ps. The dynamics of the two slower processes are slower than that of bulk water relaxation by factors of 47 and 6, respectively. The amplitudes of all three relaxation processes, in contrast, vary strongly with protein concentration. While the dielectric strength of bulk water, $\Delta\epsilon_D$, smoothly decreases with increasing protein concentration (Figure 4.3a), those of the two slower processes, $\Delta\epsilon_1$ and $\Delta\epsilon_2$, increase when c increases. Giving further insight into the amplitudes of the two slower relaxation processes, we have observed that their dielectric strengths show saturation at high protein concentrations.

The two slower processes originate from the cooperative molecular dynamics of water molecules in hydration layers. In these layers, water molecules are densely packed, and their orientations are thus highly cooperative, leading to a detectable slowdown in their dielectric relaxation times compared to that of bulk water. The longest relaxation time, $\tau_1 = 379 \pm 22$ ps, comes from water molecules in the tightly-bound hydration layer. These water molecules have strong and direct hydration bonds with the protein surface. The loosely bound water molecules, having indirect contacts or weaker hydration interactions with the protein surface, relax with the intermediate relaxation time, τ_2 , of 45 ± 11 ps.

The protein hydration structure is not only quite thin, compared to the size of the protein, but also heterogeneous on the scale of several water layers around the protein. To focus on the dielectric response of water in hydration layers, the dielectric spectra of the bound water in the MHz to GHz frequency range have been obtained by subtracting the contribution of bulk water in solution characterized by relaxation time, τ_D , and the dielectric strength, $\Delta\epsilon_D$, from the total dielectric response, $\epsilon_{\text{sol}}^*(\nu)$. Using this approach, we have obtained dielectric response of tightly-bound and loosely-bound hydration water for several BSA solutions (Figure 4.2b). This procedure reveals that dispersion and loss curves clearly exhibit relaxation processes of hydration water. The dielectric response at THz frequencies of BSA solutions is complex, including the dielectric response of collective motions of BSA proteins, and the librational and vibrational processes of water. As discussed below, we have analyzed the dielectric response of protein solutions at high frequencies separately, using the effective-medium approximation and MD simulations.

Information on the hydration volume, including the number of water molecules affected by the protein, can be obtained from the weighted contribution of each relaxation process. The addition of proteins to water alters the structure and dynamics of surrounding water molecules. This process leads to a cooperative rearrangement of the hydration-bond network of bulk water. The lowering of the amplitude of the dielectric response for bulk water with increasing protein concentration comes from two main factors: (i) the presence of proteins and (ii) water molecules in hydration shells. With the presence of proteins, the concentration of water in the solution is lower than the pure water, thus, reducing the dielectric response. We have calculated the dielectric response of the solution under the assumption that all water molecules in the solution participate in the τ_D relaxation process as in pure water, which is the “ideal bulk water” (the continuous green line in Figure 4.3a). However, the dielectric response of bulk water, $\Delta\epsilon_D$, in the protein solution from our measurements is lower, indicating that not all water molecules in the solution relax via the τ_D relaxation mode. As discussed above, the water molecules in hydration shells have much longer relaxation times. When the protein concentration increases, the fraction of hydration water increases, resulting in a lower dielectric response of the solution. For a given protein concentration, we employ this method to estimate the number of water molecules residing in all hydration shells by comparing the measured dielectric response of bulk water in the protein solution to the calculated dielectric value when all water molecules in the solution are treated as bulk water. This method has been used frequently in the literature [75, 102, 105, 108], and the number of water molecules in hydration shells per protein, termed hydration number, is given by:

$$N_{\text{hyd}}(c) = \frac{c_w - \frac{\Delta\epsilon_w}{\Delta\epsilon_{\text{pure}}} c_{\text{pure}}}{c} \quad (4.7)$$

where c_w is the molar concentration of water in the solution, and $c_{\text{pure}} = 55.35 \text{ M}$ is the molarity and $\Delta\epsilon_{\text{pure}} = 73.25$ is the dielectric strength of pure water at 25 °C [16, 55].

We have calculated the number of water molecules per protein that no longer participate in the relaxation process of bulk water due to the hydration effect. Our analyses show that $N_{\text{hyd}} = 3500 \pm 200$ for BSA solutions when the protein concentration is lower than 3 mM, and then starts to decrease as the protein concentration increases further (Figure 4.3a, inset). When the protein concentration is low, the solution is dilute and the average distance between proteins is much larger than the thickness of hydration shells. In this case the hydration shell is solely determined by water-protein interactions, and the hydration number, N_{hyd} , is thus, to the first approximation, independent of protein concentration. The estimation of N_{hyd} indicates that less than 3 layers of water molecules surrounding the protein surface are affected by the protein at low concentrations. Using MD simulations below, we have estimated about 4100 water molecules within 7.0 Å from the protein surface, which is less than 3 layers of water molecules around the protein. When the protein

concentration increases to a certain level, hydration shells start to overlap, and proteins aggregate in small equilibrium clusters, resulting in a decrease of the hydration number. A similar trend of the hydration number has been reported in dielectric measurements of lysozyme [102] and micelles [75] in a wide range of concentrations. The small-angle neutron scattering for DPC micelles has also indicated a decrease of micellar hydration number with increasing concentration as surfactants become more closely packed and compete with one another for space within the micellar arrangement [109].

4.3.2. Terahertz Spectroscopy

With a higher frequency, terahertz radiation has been used to probe vibrational modes typically involving collective atomic motions of macromolecules, which include both inter- and intramolecular interactions. Terahertz spectroscopy of biomolecules in aqueous environments, thus, provides an important approach for identifying their global and transient molecular structures as well as directly assessing hydrogen-bonding and other detailed environmental interactions [35]. However, a significant challenge in obtaining terahertz dielectric spectra of aqueous biomolecular solutions is the strong absorption of water in the spectral range of 0.5 – 10 THz. Using our high resolution and high dynamic terahertz frequency-domain spectroscopy, we have determined the absorption and refractive index spectra of protein solutions along with that of pure water (Figure 4.1). The absorption and the refractive index data indicate strong frequency dependence, increasing and decreasing with increasing frequency, respectively. It has been shown that the most prominent effect of adding solutes to water is a monotonic decrease in absorption with increasing solute concentration [32, 35, 101]. This is primarily due to the fact that the highly absorbing solvent is replaced by the solutes having lower absorption at THz frequencies.

As frequently discussed in the literature, the influence of protein-water interactions extends beyond the tightly-bound hydration layer, causing changes to the hydrogen-bonding network, and thus resulting in a strong dependence of the THz absorption on protein concentration [35, 42, 45, 100, 102, 110]. As a first approximation, we can consider protein solutions as a two-component system with BSA proteins and surrounding bulk water molecules. The total absorption of the solution as a weighted average of its two constituents is given by:

$$\alpha_{\text{sol}} = (\alpha_{\text{wat}}V_{\text{wat}} + \alpha_{\text{BSA}}V_{\text{BSA}})/V_{\text{sol}} \quad (4.8)$$

where α_{sol} , α_{wat} and α_{BSA} are absorption coefficients of the solution, water, and BSA protein, while V_{sol} , V_{wat} and V_{BSA} are their volumes, respectively.

Following this approach, the absorption of proteins in a solution can be determined by subtracting the absorption of water from the total spectrum. We have measured the

absorption coefficient of BSA solutions with concentrations from 0.038 mM (dilute) to almost the saturating level of 4.656 mM, at different frequencies of 0.32 THz and 1 THz (Figure 4.4a). During the sample preparation, we determined accurately the volume of water in protein solutions, thus we can calculate the absorption coefficient of water in protein solutions (solid lines in Figure 4.4a). As can be seen from Figure 4.4a, the measured absorption is lower at 0.32 THz and higher at 1 THz than the calculated absorption of water in BSA solutions. It means that the absorption of BSA proteins in water is negative at 0.32 THz and positive at 1 THz. In order to confirm the measurements, we have measured the absorption of BSA solutions as a function of frequency. The absorption of BSA proteins in water turns out to be negative in a frequency window from 50 GHz to 650 GHz for several protein concentrations (Figure 4.4b). A similar observation has been reported for lysozyme in water [35].

This unphysical result has previously been explained by the fact that the hydrophilic surface of a protein interact with water and bind some water molecules adjacent to them [35]. Therefore, the water molecules having strong hydrogen bonds with the protein surface cannot take part in the dipole relaxation, or in other words, they do not contribute to the absorption of bulk water in the THz frequency window. We note that the binding to the hydrophilic surface of water molecules does not necessarily mean that these water molecules are immobile. Instead, these water molecules form a tightly-bound hydration shell around a protein, and their dynamics are slower than those of bulk water molecules. Assuming the minimum of the curves in Figure 4.4b is zero, we can estimate the number of water molecules removed from bulk water. Using this method, the “lost” solvent corresponds to 750 ± 75 water molecules bound to a BSA protein (equivalent to 0.20 ± 0.02 g of water per gram of BSA protein). This value is less than a single layer of water molecules that fully cover the surface of a BSA protein. The changes of absorption at the THz frequencies with BSA concentrations follow the “Beer’s law,” in which the measured absorbance is proportional to the protein concentration. The observation suggests that the number of water molecules in the tightly-bound hydration layer of a BSA protein is independent of protein concentrations over the entire range investigated here. The assumption that the minimum absorbance of proteins is precisely zero may be not convincing, thus the above estimation yields the lower-bound of water molecules in the tightly-bound hydration shell.

In dealing with a highly heterogeneous system, we have reported both absorption and refractive index or the complex dielectric response of protein solutions with high precision, rather than assuming that overall absorption of a solution is the sum of the absorption of its constituents. The analysis based on only absorbance measurements, as we have noted above, may fail in spectral regions for which the refractive index of the solvent changes very rapidly with frequency (Figure 4.1a). With simultaneous measurements of the absorbance and refractive index, we can apply effective-medium methods to extract the dielectric response of the solute: hydrated BSA proteins.

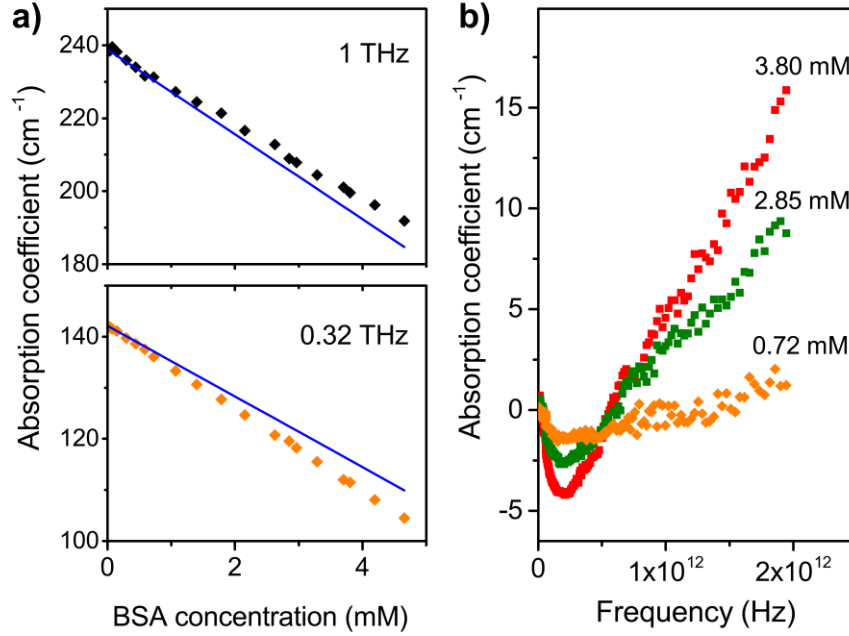


Figure 4.4: The THz absorption of hydrated BSA provides the low-frequency vibrational dynamics of proteins in water. **(a)** The THz absorption coefficient of BSA with no correction for the hydration shell of the protein (absorption of water in solutions is subtracted from solution absorption) reveals negative absorption, which is unphysical. Data points represent the experimental data, whereas solid lines show the calculated absorption reduction due to the exclude volume of the protein. Specifically, the absorption of BSA proteins in solutions shows negative absorption at 0.32 THz (lower) and positive absorption at 1 THz (upper). **(b)** The absorption spectra of BSA proteins in water show negative absorption in the range from 50 GHz to 650 GHz for several protein concentrations [103].

A protein solution is a mixture of water and protein, each of which possesses its own complex dielectric constant, $\epsilon_{\text{wat}}^*(\nu)$ and $\epsilon_{\text{pro}}^*(\nu)$, respectively. The complex dielectric response of the protein solution, $\epsilon_{\text{sol}}^*(\nu)$ has been determined from the experimental observables of absorption and refractive index, α and n , respectively (Figure 4.1b). We assume that (i) proteins in solution are spherical with a radius of R_p and have a spherical shell containing tightly-bound water with a thickness of d ; (ii) water molecules in the tightly-bound hydration shell are a part of the hydrated protein; and (iii) water outside the hydrated proteins has the same dielectric property as that of pure, bulk water. Note that the spherical shell of tightly-bound water can be a fraction of one monolayer of water molecules around the protein. Because the size of hydrated proteins is orders of magnitudes smaller than the wavelengths of the incoming electromagnetic radiation, the material can be treated as a homogeneous substance with an effective-dielectric function using the effective-medium approximation of Bruggemann [111] which effectively treats both low and high concentration mixtures. (Note: the Maxwell Garnet [112], and the Wagner-Hanai

approximations [47, 113] are for the low concentration limit of the Bruggeman approximation). The composite medium is determined from:

$$f_{\text{hp}} \frac{\varepsilon_{\text{hp}}^* - \varepsilon_{\text{sol}}^*}{\varepsilon_{\text{hp}}^* + 2\varepsilon_{\text{sol}}^*} + (1 - f_{\text{hp}}) \frac{\varepsilon_{\text{wat}}^* - \varepsilon_{\text{sol}}^*}{\varepsilon_{\text{wat}}^* + 2\varepsilon_{\text{sol}}^*} = 0 \quad (4.9)$$

where $\varepsilon_{\text{hp}}^*$ is the complex dielectric response of hydrated proteins described as the process of adding water to dry proteins; $\varepsilon_{\text{wat}}^*$ is the complex dielectric response of water; $f_{\text{hp}} = (N_p/V)(4\pi/3)(R_p + d)^3$ is the volume fraction of hydrated proteins, and N_p/V is the concentration of proteins in solution. The information provides insights into the protein dynamics as well as the number of water molecules in the tightly-bound hydration shell.

Employing the Bruggemann effective-medium analysis, we found that each BSA protein captures a tightly-bound hydration shell composed of 1150 ± 95 water molecules. Unlike the absorbance-based method used above and in prior literature [42, 45, 100], this method of estimating the size of the tightly-bound hydration shell requires only the well-founded assumption that the protein's absorption falls to zero at zero frequency [35]. The value of 1150 water molecules for the THz-defined hydration shell corresponds to a sub-monolayer on the surface of a BSA protein. Specifically, if we approximate BSA as a sphere with a diameter ~ 4 nm, then a single layer of water fully covering its surface contains ~ 1500 water molecules. Using the number of water molecules in the tightly-bound hydration shell related to the scaled filling factor and the measured $\varepsilon_{\text{wat}}^*(\nu)$ and $\varepsilon_{\text{sol}}^*(\nu)$, we have obtained the dielectric spectra of hydrated BSA proteins in several solutions (Figure 4.5). It should be noted that the hydration number calculated from the effective-medium approximation is lower than that from the GHz dielectric relaxation measurements which determine the total number of water molecules affected by the protein. This is expected as the effective-medium approximation yields the number of water molecules that have strong hydrogen bonds with the protein. These water molecules become an integral part of the protein and cannot move easily (but are not completely immobile); they form the tightly-bound hydration layer.

4.3.3. Molecular Dynamics Simulations

To provide a molecular-level picture of the dynamics and structure of hydration shells as well as the collective motions of proteins in solution, we have conducted MD simulations for BSA proteins in water. The combination of MD simulations with the MHz to THz spectroscopy leads to a microscopic level understanding of the coupled protein-water dynamics.

The MD simulations were performed using the GROMACS package (version 5.1.4) with cubic periodic boundary conditions. The gromos54a7 force field and SPC/E water

model were employed. The system was equilibrated during the first 200 ps in NPT ensemble at constant pressure (1 bar) and temperature (298 K) using Parrinello-Rahman barostat and V-rescale thermostat, and then followed by a 40 ns trajectory in a constant NVT (canonical) ensemble. The equations of motion were integrated with a time step of 2 fs, and trajectories were saved every 20 fs. Bond lengths were constrained using the LINCS algorithm. Coulombic and Lennard-Jones interactions were truncated at 1.4 nm. Long-range electrostatic interactions were calculated using the particle mesh Ewald method with an order of 4. GROMACS tools were used for data analyses. The vibrational density of states (VDoS) in the THz frequency range was calculated from the last 2 ns of trajectories using the velocity autocorrelation tool.

The tightly-bound hydration shell of proteins can be identified by examining the average density of water molecules around the protein surface. In particular, we consider oxygen and nitrogen atoms on the protein surface and calculate the density of water molecules as a function of the water-protein distance. The result is a radial density function (RDF) of water (Figure 4.5a, inset). From the water RDF, we are able to extract the number of water molecules in the hydration shells of a BSA protein. The first peak of the RDF is at 3.5 Å, while the second peak occurs around 5.5 Å. By taking a time-average of the number of water molecules within the first peak of the RDF, we have determined that the number of water molecules having strong interactions with protein surface is 1230 ± 75 . This value is in excellent agreement with the number of water molecules (1150 ± 95) in the tightly-bound hydration shell estimated from the dielectric spectroscopy at THz frequencies using the effective-medium approximation. Using the same method, we can estimate 4100 ± 75 water molecules within 7.0 Å from the protein surface.

To resolve the dynamics and the total volume of water affected by the protein, we have employed GROMACS tools to analyze the rotational (reorientation) autocorrelation functions of water molecules. Such analyses can provide information of hydration shells including how far out into the solvent the influence of the protein can reach, and how the water dynamics is affected. Using simulation data for trajectories that are 40 nanosecond long, rotational autocorrelation functions of water using blocks of 1 ns were calculated and averaged. To monitor the dynamics of water around the protein at a large distance from the protein, we have performed analyses for water within different thicknesses from the protein surface. The rotational autocorrelation functions based on the first Legendre polynomial (P_1) for water molecules within 3.5, 5.5 and 9.0 Å from the protein surface, respectively, are given in Figure 4.5a.

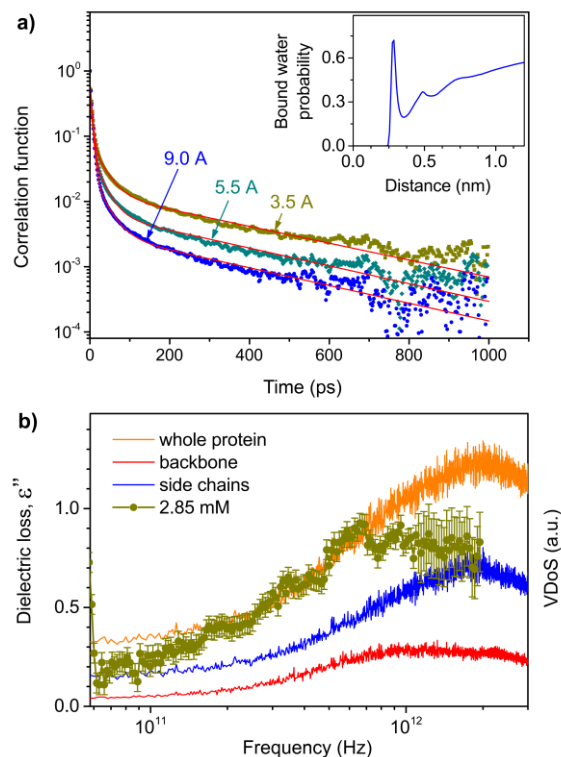


Figure 4.5: Dielectric spectra of hydrated BSA proteins in the THz frequencies and rotational autocorrelation functions, $P_1(t)$, of water providing insight into the collective motions of hydrated proteins and the dynamics of water molecules around protein surfaces. **(a)** Rotational autocorrelation functions of water molecules within 3.5, 5.5 and 9.0 Å from protein surfaces indicate three distinct dynamics corresponding to those of bulk water, tightly- and loosely-bound water around proteins, respectively. The solvent radial distribution function (upper inset) allows us to extract the number of water molecules in the tightly-bound hydration shell of a hydrated protein. **(b)** The dielectric loss spectrum (dark yellow symbols) of hydrated BSA proteins at 25 °C is extracted from the effective-medium approximation. The VDoS calculations for the side chains (blue curve), backbone (red curve), and whole protein (orange curve) have a broad peak at 1.6 THz [103].

The rotational autocorrelation functions of water around proteins can be fitted as superpositions of three exponential decay processes. Specifically, relaxation time constants of 6.85 ± 0.85 , 39 ± 9 ps, and 335 ± 50 ps were obtained for all three thicknesses, respectively. The amplitudes of the three processes, in contrast, vary strongly with the water thickness. The fastest relaxation time arises due to the bulk water in the volume around protein, that has been observed previously by nuclear magnetic resonance (NMR) [114], pump-probe experiments [115], and our dielectric relaxation spectroscopy [16]. The slower relaxation times of 335 ± 50 ps and 39 ± 9 ps align well with the experimental values of 379 and 45 ps for tightly- and loosely-bound water, respectively. These water molecules relax more slowly because of their hydrogen bonds and other intermolecular

interactions with the protein surface [116]. When the thickness of water from the protein surface is larger than 9 Å or 3 water layers from the protein surface, the contribution to the rotational autocorrelation function mainly comes from bulk water. This finding is in agreement with our estimation for the total number of water molecules ($\sim 3500 \pm 200$) affected by the protein in dielectric response measurements.

The contribution of the collective dynamics of BSA proteins in an aqueous solution to the THz spectra can be obtained by computing the vibrational density of states (VDoS) spectra using MD data (Figure 4.5b). The VDoS calculations for the side chains (blue curve), the backbone (red curve), and the whole protein (i.e., the side chains and the backbone; orange curve) all show a broad peak at 1.6 THz, which is consistent with the experimental spectra (dark yellow symbols). The analyses allow us to delineate contributions from different motions. The MD data show that the motion of the protein side chains has a larger contribution to the density of states than the backbone, although both increase with frequency, as is observed in the experimental THz spectra. The higher flexibility of side chains in this frequency window agrees with MD calculations for lysozyme proteins in water using analysis of root mean square fluctuations (RMSFs) [73]. Thus, our analyses reveal that the collective motions in this frequency window primarily arise from side-chain torsional oscillations or hindered motions about terminal single bonds [73]. Both the THz spectrum and the backbone VDoS have maximum intensity around 1 THz, which supports the assertion that THz dielectric spectroscopy is sensitive to the large-domain motions of proteins in solution.

4.4. Conclusion

Employing our high-precision dielectric spectroscopy in a wide frequency range, from 100 MHz to 2 THz, and MD simulations, we have demonstrated that vibrational spectra of BSA proteins in water exhibit a broad peak at 1.2 THz, and water molecules in hydration shells of the protein reveal retarded reorientation dynamics relative to bulk water. The MHz to GHz dielectric spectroscopy reveals three main relaxational processes of water in aqueous BSA solutions, including the tightly-bound water with a reorientation time of 379 ps, loosely-bound water with a relaxation time of 45 ps, and bulk water with a relaxation time of 8 ps. The hydration number or the total amount of hydration water molecules affected by the presence of a BSA protein has been determined to be ~ 3500 , including both tightly- and loosely-bound water. When the protein concentration is higher than 3 mM, hydration shells start to overlap, and proteins aggregate in small equilibrium clusters, resulting in a decrease of the hydration number.

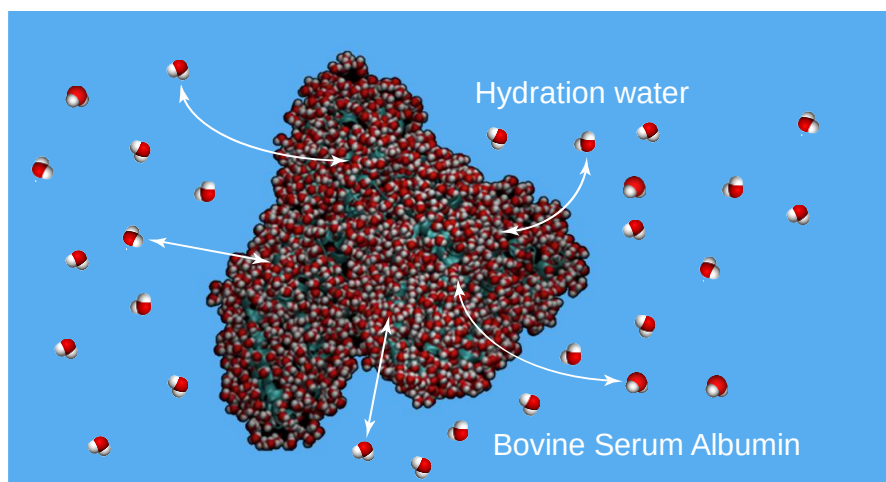


Figure 4.6 Schematic representation of BSA in liquid water interacting with bulk water molecules [103].

Using the effective-medium approximation for the dielectric measurements at the THz frequencies, we are able to extract the number of tightly-bound water molecules, which is about 1150 molecules per protein, as well as the collective vibrational modes of hydrated BSA proteins. MD simulations yield results in excellent agreement with experiments. In particular, simulations show that there are ~ 1230 water molecules directly hydrogen-bonded to the surface of a BSA protein, and the total hydration layer has a mean thickness of ~ 7 Å.

Our results indicate that the effects of a BSA protein in water is beyond the first hydration shell but there is no long-range effect on bulk water structure. MD simulations also reveal that the dominant contribution to the THz peak in the dielectric spectra comes from large-domain motions of BSA proteins.

Chapter 5

Dynamics of Zwitterionic Micelles and Their Hydration Waters

This chapter was adapted with only minor changes from the manuscript:

“Reprinted with permission from D. K. George, A. Charkhesht, O. A. Hull, A. Mishra, D. G.S. Capelluto, K. R. Mitchell-Koch, and N. Q. Vinh (2016). New insights into the dynamics of zwitterionic micelles and their hydration waters by gigahertz-to-terahertz dielectric spectroscopy. The Journal of Physical Chemistry B, 120(41), 10757-10767. Copyright 2016 American Chemical Society.”

Gigahertz-to-terahertz spectroscopy of macromolecules in aqueous environments provides an important approach for identifying their global and transient molecular structures, as well as directly assessing hydrogen-bonding. We report dielectric properties of zwitterionic dodecylphosphocholine (DPC) micelles in aqueous solutions over a wide frequency range, from 50 MHz to 1.12 THz. The dielectric relaxation spectra reveal different polarization mechanisms at the molecular level, reflecting the complexity of DPC micelle-water interactions. We have made a deconvolution of the spectra into different components and combined them with the effective-medium approximation to separate delicate processes of micelles in water. Our measurements demonstrate reorientational motion of the DPC surfactant head groups within the micelles, and two levels of hydration water shells, including tightly- and loosely-bound hydration water layers. From the dielectric strength of bulk water in DPC solutions, we found that the number of waters in hydration shells is approximately constant at 950 ± 45 water molecules per micelle in DPC concentrations up to 400 mM, and it decreases after that. At terahertz frequencies, employing the effective-medium approximation, we estimate that each DPC micelle is surrounded by a tightly-bound layer of 310 ± 45 water molecules that behave as if they are an integral part of the micelle. Combined with molecular dynamics simulations, we determine that tightly-bound waters are directly hydrogen-bonded to oxygens of DPC, while loosely-bound waters reside within 4 Å of micellar atoms. The dielectric response of DPC micelles at terahertz frequencies yields, for the first time, experimental information regarding the largest-scale, lowest frequency collective motions in micelles. DPC micelles are a relatively simple biologically relevant system, and this work paves the way for more insight in future studies of hydration and dynamics of biomolecular systems with gigahertz-to-terahertz spectroscopy.

5.1. Introduction

Biological membrane components are amphipathic molecules, such as surfactants and lipids that can undergo surface and interfacial adsorption when dissolved in an aqueous solution. When surfactant molecules are dispersed in water, they aggregate to form micelles above a critical concentration, with the hydrophobic tails making up the core and hydrophilic head groups forming the shell. The separation of the hydrophobic and hydrophilic regions of micelles has been utilized extensively as an excellent tool to mimic biological environments and activities of lipid membranes [117-119]. Among a large variety of amphipathic molecules available for purifying and characterizing membrane proteins, the zwitterionic surfactant dodecylphosphocholine (DPC) (Figure 5.1) forms spherical micelles with aggregation numbers of about 56 ± 5 at a concentration greater than the critical micelle concentration of 1 mM in aqueous solution [120, 121]. The zwitterionic surfactants are electrically neutral, but the charge they carry in the phosphocholine head group does influence the hydrophilic properties. Many zwitterionic surfactants have been used as model membrane systems because of characteristics such as the ability to form stable micelles [122, 123], and the ability to bind to peptides and proteins while mimicking the anisotropic environment of a lipid membrane [122-125]. DPC micelles have a simpler structure [126] than most proteins, so studying the dynamics of micelles and their hydration layers offers an opportunity to determine processes that are not related to the specific structural vibrations of proteins. The outer micelle's hydrophilic surface is in contact with water, and thus, the system offers an opportunity to investigate the interaction of water with hydrophilic, biologically-relevant surfaces.

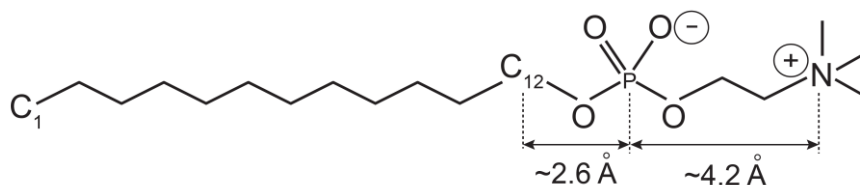


Figure 5.1: Chemical structure of DPC showing the numbering used in the text [75].

The dynamics of water in the hydration layer around proteins and other biomolecules play a crucial role in different aspects of biological processes. Some studies have concluded that water molecules in the hydration layer are rigidly attached to surfaces of molecules, resulting in an increase in the effective volume of the molecules [127]. On the other hand, evidence for the dynamic nature of the hydration layer is also abundant [128], with some suggesting that there are fast and slow dynamic processes within the hydration layer. Some of the dynamical processes in proteins have been suggested as

solvent slaved motions [129]. The significance of the hydration layers cannot be overstated in biological process and reactions, as they control the structure and function of biological systems [72]. There are many experimental techniques that allow the investigation of dynamics and structure of hydration water on a biomolecular surface. These include time-resolved fluorescence [31], dielectric relaxation spectroscopy at gigahertz (GHz) frequencies [130-133], nuclear magnetic resonance [134], X-ray crystallography [135], neutron scattering [136], and infrared spectroscopy [137]. Among these techniques, dielectric spectroscopy from GHz to terahertz (THz) frequencies and computational techniques are advantageous for investigating the dynamics of water in confined systems, including interfacial or restricted environments, providing information on the hydrogen bonding, diffusion, and reorientation of water around DPC micelles as well as the dynamics of micelles themselves. The structural and dynamical properties of water in the relatively simple structure of DPC micelles will shed light on hydration dynamics in biological systems.

THz vibrational modes typically involve the low frequency, collective atomic motions of macromolecules, which include both inter- and intramolecular interactions. Thus, THz spectroscopy of biomolecules and lipid layers in aqueous environments provides an important approach for identifying their global and transient molecular structures as well as directly assessing hydrogen-bonding and other detailed environmental interactions [35, 49, 101]. However, a significant challenge in obtaining THz dielectric spectra of aqueous biomolecules and lipid layers is the strong absorption of water in the spectral range of 0.5 – 10 THz. The dielectric relaxation of surfactant micellar solutions have been reported [130, 138, 139], but only below 89 GHz, hence dealing with the fluctuation of ion distribution and rotational motion of polar molecules. Advances in GHz to THz technology call for a more thorough study of the dielectric response of such simple systems. Such a study can act as an important step in understanding the behavior of more complex biomolecular systems in the GHz to THz frequency range. Micellar solutions have been extensively used in microwave and THz spectroscopy, especially for studies on nano-confined water [140] in the form of water dispersed in reverse micellar solutions. THz spectroscopy of protein-containing reverse micelles has also been investigated in the past as an alternative approach for probing collective vibrational motions in solution [141]. Recent developments in diode based frequency multipliers have improved the accuracy of THz measurements by several orders of magnitude, which allows for high-precision measurements of the strong absorption of aqueous solutions. In this chapter, we investigate interactions of zwitterionic DPC micelles with water, using a spectrometer with a frequency range from 0.05 GHz to 1.12 THz.

5.2. Materials and Methods

5.2.1. Materials and solution preparation

Dodecylphosphocholine (DPC), purchased from Anatrace (Cat. No. 29557), was used to prepare micellar solutions. DPC (m.w. = 351.5) was dissolved in deionized water to form micellar solutions with concentrations ranging from 50 to 800 mM. Accurate determination of the molarity of the solutions as well as the volume filling factor of DPC in solution was critical in our calculations. The solutions were prepared by weighing and measuring the volume after dissolving DPC in deionized water for several times to obtain an accurate value of the partial specific volume. The concentration of DPC in solutions was above the critical micelle concentration of 1 mM [120, 121, 142], at which the liquid forms nearly spherical micelles with aggregation number of 56 ± 5 [120, 121]. Results focus on the dynamics of water as well as the dynamics of DPC spherical micelles in solution. The chemical structure of DPC with the numbering used in the text is shown in the Figure 5.1.

5.2.2. Complex permittivity spectra

We have collected complex dielectric response spectra from DPC micellar solutions in a large range of frequencies, from 50 MHz to 1.12 THz, using two different methods. From 50 MHz to 50 GHz, a vector network analyzer (PNA N5225A) was combined with a dielectric probe (HP 85070E) and a transmission test set to measure the low frequency dielectric response (50 MHz – 5 GHz) of solutions. The cell of the transmission set consists of a coaxial line/circular cylindrical waveguide transition containing solutions. Air, water, and a shorting block were used as the standards for calibration of the dielectric probe. The real and imaginary parts of the dielectric constant were obtained directly from the system. The sample was kept in a sample cell made of anodized aluminum and the temperature was set at 25°C and controlled with an accuracy of $\pm 0.02^\circ\text{C}$ using a Lakeshore 336 temperature controller.

The dielectric response of the samples at high frequency were studied using a GHz-to-THz spectrometer based on the above vector network analyzer together with frequency multipliers from Virginia Diodes, spanning the frequency range from 60 GHz to 1.12 THz. The setup is capable of simultaneously measuring intensity and phase over a large effective dynamical range of fifteen orders of magnitude [16]. Samples were kept in a home built variable path-length cell with submicron (~ 0.08 microns) precision in changing thickness. The temperature of the sample is controlled with the previously mentioned temperature controller. The sample cell was built with anodized aluminum to ensure thermal stability of solutions. For each frequency, we measured 200 data points of the intensity and phase shift of solutions as a function of the path-length. The absorption and refractive index of solutions at each frequency were determined from the best fit of the intensity and phase

data to the sample thickness. The very high dynamic range of the frequency extenders, together with the precise sample thickness controller, allows us to obtain the most highly precise and accurate GHz-to-THz dielectric response spectra reported so far for this frequency range (Figure 5.2).

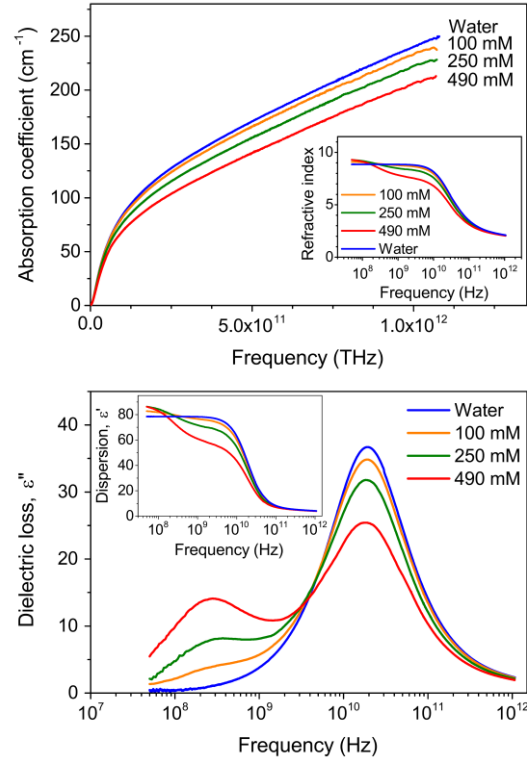


Figure 5.2: The interaction of DPC micelles with GHz to THz radiation provides insight into the liquid's dynamics over picosecond to nanosecond timescales. (top) The absorption spectra of both DPC micellar solutions and pure water rise with increasing frequency. The refractive indexes (upper inset) of DPC micelles and water, in contrast, decrease with increasing frequency. (bottom) The dielectric loss and the dielectric dispersion spectra (lower inset) from DPC aqueous solutions and pure water are obtained from absorption coefficient and refractive index measurements. Data were collected at 25°C [75].

With simultaneous measurements of the absorption and refractive index, the complex refractive index of a material can be expressed as

$$n^*(\nu) = n(\nu) + i\kappa(\nu) \quad (5.1)$$

where ν is frequency, $n(\nu)$ is the refractive index of the solution, and $\kappa(\nu)$ the extinction coefficient of the solution. $\kappa(\nu)$ is related to the absorption coefficient, $\alpha(\nu)$, by $\kappa(\nu) =$

$c\alpha(\nu)/(4\pi\nu)$ with c being the speed of light. Similarly, the complex dielectric constant of a material can be expressed as

$$\varepsilon^*(\nu) = \varepsilon'(\nu) + i\varepsilon''(\nu) \quad (5.2)$$

where $\varepsilon'(\nu)$ and $\varepsilon''(\nu)$ are the dielectric dispersion and dielectric loss components. Since our experiment can simultaneously measure both the absorption and refractive index of a material, $\varepsilon_{\text{sol}}^*(\nu)$, the complex dielectric response can be calculated from the following relations:

$$\begin{aligned} \varepsilon'_{\text{sol}}(\nu) &= n^2(\nu) - \kappa^2(\nu) = n^2(\nu) - (c\alpha(\nu)/4\pi\nu)^2, \\ \varepsilon''_{\text{sol}}(\nu) &= 2n(\nu) \cdot \kappa(\nu) = 2n(\nu)c\alpha(\nu)/4\pi\nu. \end{aligned} \quad (5.3)$$

From our absorption and refractive index measurement (Figure 5.2, top), we have determined the dielectric spectra of DPC micellar solutions (Figure 5.2, bottom).

5.2.3. Molecular dynamics simulation details

Molecular dynamics (MD) simulations have been performed using the GROMACS package (version 4.5.3) [143] with cubic periodic boundary conditions. The force field for DPC used in this work was parametrized by Abel *et al.* [126] for GROMOS54A7 with SPC/E water model [144]. For initializing MD simulations, the topology file and coordinates of an equilibrated DPC micellar aggregate (comprised of 54 surfactant molecules) were acquired from Abel's website [145] and DPC force field parameters were used as published by Abel *et al.* [126]. First, the system was equilibrated for 50 ns at constant pressure (1 bar) and temperature (298 K) using Berendsen's barostat [146] and V-rescale thermostat [68]. Next, a 60.5 ns simulation was run at constant volume in a cube with length 7.44 nm, for data collection in a canonical ensemble using the Nosé-Hoover [147, 148] thermostat at $T = 298$ K. Water and surfactant were coupled separately, with a thermostat time constant of 0.4 ps. A time step of 2 fs was used to integrate the equations of motion. Bond lengths for the 54 DPC molecules were constrained using the LINCS algorithm [143], with the SETTLE algorithm for the 12,794 water molecules [149]. Electrostatic interactions were calculated using the particle mesh Ewald method [74], with an order of 4. Cutoffs of 1.4 nm were used for Coulombic and Lennard-Jones interactions. Analysis of MD data was carried out using GROMACS analysis tools and Octave [150]. The THz spectrum was calculated from MD simulations through a density of states (DoS) calculation (`g_dos` in the GROMACS package), which is acquired from the velocity autocorrelation function. The DoS analysis was run on the DPC surfactants in the micelle only (no waters contributing).

5.3. Results and Discussion

5.3.1. Low frequency dielectric response (50 MHz to 50 GHz)

The dielectric response of polarization mechanisms in micellar solutions is still a matter of discussion, which requires more data collection on cationic, anionic, and zwitterionic surfactants. Complex dielectric responses of ionic surfactant micelles have been reported previously for cationic (hexadecyltrimethylammonium bromide - CTAB) [130] and anionic (sodium dodecyl sulfate – SDS) [139] micellar solutions (aqueous). In this chapter, we have reported for the first time the dielectric response of zwitterionic DPC micellar solutions. The general observation for all three of these micelles is that a dielectric loss peak around 300 MHz (0.6 ns) is absent from the pure water spectrum, but present in micellar solutions. The amplitude of the dielectric loss increases with increasing micellar concentration. The responsible polarization mechanism at 0.6 ns was proposed to be due to the diffuse counterions [130]. It is noteworthy that the peak occurred essentially at the same frequency with similar shape and amplitude for both CTAB and SDS micelles. Later, the polarization mechanism was interpreted as the rotation of stable ion pairs [138, 151] or as hopping of counterions bound to the charged surface of the micelles [152]. However, given the similar observation for SDS, CTAB, and DPC, the fact that DPC micelles are zwitterionic, without counterions, makes this argument less likely. For DPC micelles, there will not be any electrical double layer formed by ionic clouds around micelles and hence the likelihood of a tangential counterion current, which seems to be a prerequisite for the dielectric response if we follow the argument in the above papers, is negligible. Thus, the results with the 0.6 ns timescale component in zwitterionic micelles indicate that counterions may not play an important role in the relaxation processes measured for cationic and anionic micelles. It is interesting to note the results of Tieleman *et al.*, who calculated the orientational correlation functions (P_2) of carbon atoms in the alkyl tails of surfactants in simulated DPC micelles, for comparison with NMR data [153]. They found timescales in the carbon rotation autocorrelation function on the order of hundreds of picoseconds. Since the environments of hydrophobic tails are similar within micelles, it is reasonable to expect that hydrophobic tails within different micelles may experience similar timescales of dynamics. It may also be reasonable to predict that different surfactants within micelles can experience similar reorientational (dipole moment) timescales. The reorientation of DPC surfactants within the micelle is discussed further below.

The dielectric properties of aqueous DPC micellar solutions at GHz frequencies show a complex behavior that originates from different polarization mechanisms at the molecular level (Figure 5.2, bottom). The main loss peak frequency centered at ~19 GHz (~8.27 ps) remains virtually unchanged. This value was found to be the same as for pure water.[16, 56, 59, 107] In this frequency range, the dielectric response of aqueous micellar

solutions mainly has contributions from (i) the motion of macromolecules, *i.e.*, the rotation of micelles; (ii) the motion of surfactants within the micelle (*i.e.*, undulation and motion of head groups); (iii) hydration waters in the interfacial region surrounding DPC micelles (dipoles of water molecules in hydration shells); and (iv) the orientational polarization of bulk water molecules (water dipoles). The dielectric response of the orientational relaxation of molecular dipoles for macromolecules with the size of ~ 15 kDa, such as the entire DPC micelle, is typically in the range of 1 to 30 MHz [102]. We do not focus on these measurements in this report. The dielectric response of the orientational polarization of bulk water has been well established [16, 59]. However, the contribution of water in hydration shells of biomolecules is complicated. For example, the dielectric response of water in the hydration shells of lysozyme [102] and ribonuclease A [105] in aqueous solution consists of several dispersion regions. The contribution of water in hydration shells mainly originates from two kinds of hydration water, *i.e.*, tightly- and loosely-bound water. The first hydration layer consists of water strongly interacting with the macromolecular surface. The second layer, which has weaker interactions with the macromolecular surface or is not in direct contact, consists of loosely bound water molecules that exchange with the tightly-bound water and have dynamics approaching those of bulk water.

In the low frequency region, where librational motions and inertial effects do not contribute appreciably to the dielectric response, it is sufficient to consider Debye-type relaxations to analytically present our spectra within error limits. [Note that we have employed the Havriliak-Negami equations [106] to examine our data, but it did not show better results]. We have obtained dielectric response for the motion of surfactant head groups, and the tightly-bound, loosely-bound and bulk water in the form:

$$\varepsilon^*(\nu) = \varepsilon_\infty + \frac{\Delta\varepsilon_1}{1 + i2\pi\nu\tau_1} + \frac{\Delta\varepsilon_2}{1 + i2\pi\nu\tau_2} + \frac{\Delta\varepsilon_3}{1 + i2\pi\nu\tau_3} + \frac{\Delta\varepsilon_D}{1 + i2\pi\nu\tau_D} \quad (5.4)$$

where ε_0 is the permittivity of free space. $\Delta\varepsilon_1$, $\Delta\varepsilon_2$, $\Delta\varepsilon_3$ and $\Delta\varepsilon_D$ are the dielectric strengths of each Debye process to the total relaxation for the motion of head groups on the micellar surfactant, the tightly-bound, the loosely bound, and the bulk water, respectively, while τ_1 , τ_2 , τ_3 and τ_D are their relaxation times, respectively. ε_∞ includes contributions to the dielectric response from modes at higher frequencies. The electrical conductivity of DPC micellar solutions is below the detection of our electric conductivity measurements. Thus, we do not include the *d.c.* electrical conductivity in the dielectric response in the eq. (5.4). At 25°C, the well-known rotation relaxational time, τ_D , for bulk water is typically 8.27 ps [16, 56, 59, 107, 133]. The dielectric spectra of bulk water could be formally fitted by a superposition of two or three Debye processes [16], yielding $\tau_D \sim 8.27$ ps (19 GHz), $\tau_{D2} \sim 1.1$ ps (145 GHz), and $\tau_{D3} \sim 0.178$ ps (895 GHz) at 25°C. In this frequency range up to 50 GHz, the contributions of the dielectric response of bulk

water at the high end of the frequency range are small, and can be neglected. The contributions from these modes at these high frequencies appear in the ε_∞ parameter.

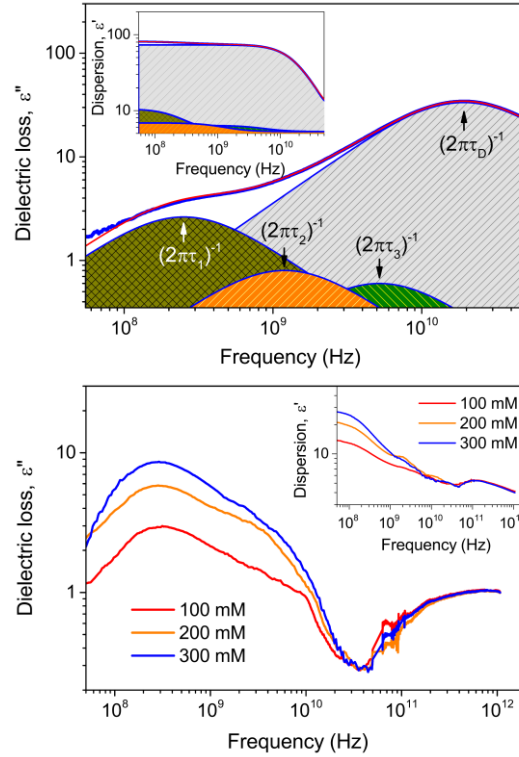


Figure 5.3: The dielectric loss and dielectric dispersion spectra of DPC aqueous solutions show relaxation processes at GHz frequencies. **(top)** The dielectric loss and dielectric dispersion **(upper inset)** spectra of 100 mM DPC in water provide insight into the dynamics of water molecules and micelles at the surface. The red curves are fits of the real and the imaginary components of the complex dielectric response. **(bottom)** The dielectric loss and dielectric dispersion spectra **(lower inset)** of the motion of surfactant head groups, the tightly- and loosely-bound water for several DPC micellar solutions have been obtained by subtracting the well-defined relaxation contribution of bulk water from the total spectrum. This procedure revealed their features in relaxation processes [75].

Using this method, we simultaneously fit the dielectric dispersion, ε' , and loss, ε'' , with the same set of free parameters. Eight parameters in eq. (5.4) are varied simultaneously and the relaxation time for bulk water, τ_D , is held fixed at the literature value [16, 56, 59, 107]. Typical dielectric spectra for both dielectric dispersion, ε' , and dielectric loss, ε'' , together with their spectral deconvolution are calculated for a DPC concentration of 100 mM (Figure 5.3, top). The fit to the four-Debye model for GHz frequencies produces $\varepsilon_\infty = 5.1 \pm 0.5$, which is now within experimental uncertainty of the prior literature value [59, 107] and $\Delta\varepsilon_1$, $\Delta\varepsilon_2$, $\Delta\varepsilon_3$, $\Delta\varepsilon_D$ of 5.4 ± 0.5 , 1.1 ± 0.3 , 0.8 ± 0.3 ,

and 68.6 ± 0.3 , respectively. The dielectric loss spectrum obtained from the four-Debye model indicates four relaxation processes centered at 251 ± 15 MHz ($\tau_1 \sim 633 \pm 39$ ps), 1.38 ± 0.13 GHz ($\tau_2 \sim 105 \pm 10$ ps), 5.13 ± 0.95 GHz ($\tau_3 \sim 31 \pm 7$ ps), and 19.25 ± 0.78 GHz ($\tau_D \sim 8.27 \pm 0.35$ ps). The dominating τ_D process, the principle process for hydrogen-bonding liquids, reflects the cooperative reorientational dynamics of the dipole moment of bulk water in solution.

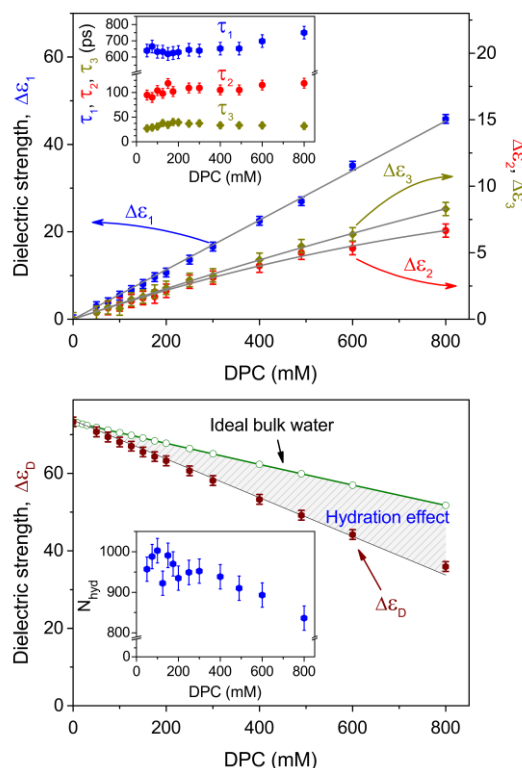


Figure 5.4: Waters' molecular-scale relaxations as a function of DPC micellar concentration, c , provides insight into their mechanistic relaxational processes. **(top)** The amplitudes of dielectric response of the motion of DPC head groups on the micellar surfactant, $\Delta\epsilon_1$, tightly-bound water, $\Delta\epsilon_2$, and loosely-bound water, $\Delta\epsilon_3$, increase with rising DPC micellar concentration. The continuous lines serve as guides for the eye. The inset to the top shows their relaxation times, τ_1 , τ_2 and τ_3 , respectively, as a function of DPC micellar concentration. **(bottom)** The dielectric strength of bulk water, $\Delta\epsilon_D$, in DPC micellar solutions decreases with increasing DPC concentration. The continuous (green) line represents the ideal bulk-water dielectric amplitude from analysis of water concentration in solutions under an assumption that all water molecules in solution contribute to the bulk water process. The inset shows the hydration number as a function of DPC micelles concentration [75].

A more generalized model-independent approach for the low-frequency part of dielectric spectra has been achieved by subtracting the well-defined τ_D relaxation

contribution from the total spectrum. The value for the dielectric strength, $\Delta\epsilon_D$, and relaxation dynamics, τ_D , obtained from the contribution of bulk water is then subtracted from the measured dielectric response, $\epsilon^*(\nu)$. We have obtained dielectric response for several DPC micellar solutions (Figure 5.3, bottom). This procedure revealed that dispersion and loss curves clearly exhibit their relaxation processes. The dielectric response at THz frequencies of DPC micellar solutions is complex, including the dielectric response of the collective motions of micelles and the librational and vibrational processes of water. As indicated below, we analyzed these dynamics in the high frequency response part separately, using the effective-medium approximation and MD simulations.

The accuracy in the evaluation of dielectric parameters, including dielectric strengths and their relaxation times, depends on the magnitude of the dielectric response, mainly on the micellar concentration. By fitting eq. (5.4) to experimental spectra, we have obtained dielectric parameters for the dielectric strengths of relaxation processes (Figure 5.4, top), and their relaxation times, respectively (inset in Figure 5.4, top), for several DPC micellar solutions. The time constants for relaxation processes are independent of micellar concentration. The amplitudes of the dielectric strengths vary strongly with rising concentration of micelles (Figure 5.4). Specifically, while the dielectric strength for bulk water in micellar solutions at $\tau_D \sim 8.27$ ps decreases with increasing DPC concentration (Figure 5.4, bottom), the dielectric strengths for slower processes increase with concentration. Further, the amplitudes for two processes, τ_2 and τ_3 , show saturation at high micellar concentrations.

The dielectric measurements provide us information about relaxation processes in DPC micellar solutions. The relaxation process at the slowest time constant cannot be attributed to the rotational process of DPC micelles because τ_1 is much smaller than the predicted relaxation timescale of macromolecules with a size of 15 kDa [102, 133]. Regarding whether this signal (633 ± 39 ps) can be attributed to water dynamics at the micellar interface, it is instructive to consider the chemical makeup of the hydrophilic head groups. The DPC surfactants are electrically neutral, and the head groups contain several heteroatoms. However, the surface of DPC micelles provides fewer hydrogen bonds when compared to a protein surface. We can expect that the relaxation times for water in hydration layers of DPC micelles are faster than those of proteins, which typically exhibit slowest relaxation times on the order from 300 to 500 ps [102, 105]. As shown in Figure 5.4, the dielectric strength for the slowest process, τ_1 , increases linearly for the whole range of DPC micellar concentrations. Thus, this relaxation has to be assigned to the rotation of the DPC surfactant, primarily, motion of the head groups. This interpretation is well supported by our dynamics studies of DPC head groups in the micellar environment by MD simulations.

The dynamics of water in aqueous DPC micellar solutions present a complex dielectric response behavior at the molecular level. As mentioned above (Figure 5.4), the

amplitudes of the dielectric strengths for faster processes, τ_2 and τ_3 , saturate at high micellar concentrations, thus these dynamics cannot be attributed to micelle-specific processes. The dynamics for these processes centered at 1 – 2 GHz (~ 105 ps) and 4 – 6 GHz (~ 31 ps), respectively, are greater than that of bulk water relaxation by factors of 13 and 4. From the behavior of dielectric strengths and the relaxation times, these processes are related to cooperative molecular dynamics in hydrated layers. The processes are highly cooperative in the densely packed hydration layers of DPC micelles, leading to a detectable slowdown in the dielectric relaxation times of bound water compared to that of bulk water. Water molecules having the relaxation time, τ_2 , of 105 ± 10 ps are in the tightly-bound hydration layer. These water molecules have a strong and direct hydration bond with DPC surfactant. The loosely bound water molecules, having indirect contacts or weaker hydration interactions with the macromolecular surface, have a relaxation time, τ_3 , of 31 ± 7 ps.

The structure of hydration shells, which are heterogeneous at the molecular level and distinct from bulk water, could be obtained from dielectric measurements. The hydration water, reflecting the total number of water molecules affected by macromolecules, can be deduced from the dielectric strength of bulk water. Therefore, this parameter is used to determine the water content in different kinds of soft materials [102, 108]. The presence of DPC micelles in aqueous solutions causes a decrease in the amplitude of the dielectric response of bulk water for two reasons: (i) The presence of the DPC micelles in solution reduces the volume of bulk water in solution, resulting in an overall lowering of the dielectric response. The continuous line (Figure 5.4, bottom) represents the dielectric strength of ideal bulk water under an assumption that all water molecules in micellar solutions participate in the τ_D relaxation process of bulk water; (ii) Water molecules have hydrogen bonds in the vicinity of micelles and these water molecules no longer contribute to the τ_D relaxation process of bulk water. We estimated how many water molecules participated in hydration shells, through a comparison of the dielectric values for the bulk water in DPC solutions and the dielectric response of the total volume of water added to the solutions as a function of the concentration, c , of micellar solutions using the Cavell equation [102, 105]:

$$\Delta\epsilon_j = \frac{\epsilon}{\epsilon + A_j(1-\epsilon)} \frac{N_A c_j}{3k_B T \epsilon_0} \frac{\mu_0^2}{(1 - \alpha_j f_j)^2}. \quad (5.5)$$

The equation connects the dielectric strength of the j th relaxation process, $\Delta\epsilon_j$, to the concentration of micelle in solutions, c , the shape parameter of the relaxing particle, A_j (for sphere $A_j = 1/3$), the thermal energy, $k_B T$, Avogadro's number, N_A , the static permittivity, ϵ , the vacuum permittivity, ϵ_0 , its permanent dipole, μ_0 , its polarizability, α_j , and the reaction field factor, f_j . Normalizing eq. (5.5) to pure water, we obtain the apparent water concentration as a function of micellar concentration, $c_w^{\text{app}}(c)$ [133]:

$$c_w^{\text{app}}(c) = \frac{\Delta\epsilon_D(c) \epsilon(0)(2\epsilon(c) + 1)(1 - \alpha_w f_w(c))^2}{\Delta\epsilon_{\text{pure}} \epsilon(c)(2\epsilon(0) + 1)(1 - \alpha_w f_w(0))^2} c_{\text{pure}} \quad (5.6)$$

where $\Delta\epsilon_{\text{pure}} = 73.25$ is the dielectric strength of pure water at 25°C [16, 56, 59, 107], $c_{\text{pure}} = 55.35$ M represents the molarity of pure water. We define the effective hydration number per micelle, $N_{\text{hyd}}(c)$:

$$N_{\text{hyd}}(c) = \frac{c_w - c_w^{\text{app}}(c)}{n \cdot c} \quad (5.7)$$

where c_w is the concentration of water in the solution, and n is the aggregation number of 56 ± 5 for DPC micelles.

Table 5.1 Relaxation times, (τ_i), and amplitudes, ($\Delta\epsilon_i$), of dielectric response of the motion of head groups on the micellar surfactant, tightly-bound water, and loosely-bound water as well as the hydration number, N , per micelle.

DPC (mM)	τ_1 (ps)	τ_2 (ps)	τ_3 (ps)	$\Delta\epsilon_1$	$\Delta\epsilon_2$	$\Delta\epsilon_3$	$\Delta\epsilon_D$	N
50	640	95	27	3.0	0.5	0.5	70.73	957
75	665	90	29	3.9	0.8	0.9	69.40	988
100	633	105	31	5.4	1.1	0.8	68.60	995
125	633	98	37	6.7	1.4	1.5	67.02	922
150	619	118	34	8.1	1.6	1.7	65.54	991
175	625	102	40	9.7	1.7	2.1	64.33	970
200	630	176	39	10.7	2.1	2.4	63.25	935
250	645	109	36	13.6	2.8	3.0	60.66	949
300	640	109	37	16.6	3.1	3.3	58.16	952
400	652	105	33	22.5	4.0	4.5	53.29	938
490	652	105	33	27.0	5.0	5.5	49.20	910
600	698	115	33	35.2	5.3	6.4	44.20	893
800	750	119	31	45.8	6.7	8.3	35.94	836

These water molecules per micelle do not participate in relaxation processes of bulk water due to the hydration effect (inset in Figure 5.4, bottom). As can be seen, the hydration number is approximately constant at 950 ± 45 water molecules per micelle in the low range of DPC concentration up to 400 mM, but it decreases at higher DPC concentration. The relaxation parameters and hydration number per DPC micelle are summarized in Table 5.1.

5.3.2. High frequency response (60 GHz to 1.12 THz)

THz spectroscopy is a new tool to study solvation effects by probing the coupled collective modes of solute and solvent [16, 35]. It is experimentally challenging due to the strong THz absorption of water. Using our high resolution and dynamic THz frequency-domain spectroscopy and a variable-thickness cell, we have determined the absorption coefficient and refractive index of DPC micellar solutions along with that of pure water (Figure 5.2). A quick glance at the absorption as well as the refractive index data indicates that these are strong functions of frequency, increasing and decreasing with increasing frequency, respectively. It is evident that the most prominent effect of addition of solvent is a monotonic decrease in absorption with increasing solvent concentration. This is primarily due to the fact that the higher absorbing solvent is replaced by the solute having a much lower absorption.

In dealing with the heterogeneous system, the dielectric response of dispersed solvent molecules in a bulk solvent is employed, rather than assuming that overall absorption is the sum of the absorption of its constituents. DPC micellar solutions are a mixture of water and DPC micelles and their complex dielectric response were determined from the experimental observables (Figure 5.2, bottom). We assume that (i) DPC micelles in solution are spherical with a radius of R_{micelles} and have a spherical hydration shell with a thickness of d ; (ii) the water molecules in the hydration shell are a part of DPC micelles; (iii) solvent outside of the DPC micelles has the same dielectric property as that of pure water. Since the size of DPC micelles with hydration water is orders of magnitude smaller than the wavelength of the probing electromagnetic radiation, the medium can be considered homogenous with an effective dielectric response. A more elegant method has been employed for the effective-medium approximation, such as the Bruggeman model [111] which effectively treats both low and high concentration mixtures or Maxwell Garnet [112], Wagner and Hanai approximations [47, 113] which are for low concentration limits. Following the Bruggeman approximation, the complex dielectric response of the solution can be determined from:

$$f_{\text{micelles}} \frac{\epsilon_{\text{micelles}}^* - \epsilon_{\text{sol}}^*}{\epsilon_{\text{micelles}}^* + 2\epsilon_{\text{sol}}^*} + (1 - f_{\text{micelles}}) \frac{\epsilon_{\text{wat}}^* - \epsilon_{\text{sol}}^*}{\epsilon_{\text{wat}}^* + 2\epsilon_{\text{sol}}^*} = 0 \quad (5.8)$$

where $\epsilon_{\text{wat}}^*(\nu)$ is the complex dielectric response of water; $\epsilon_{\text{micelles}}^*$ is the complex dielectric response of hydrated DPC micelles described as the process of forming an aggregate with hydrophilic regions in contact with surrounding solvent; $f_{\text{micelles}} = (N_{\text{DPC}}/V)(4\pi/3)(R_{\text{micelles}} + d)^3$ is the volume fraction of the micelles with hydration water, and N_{DPC}/V is the concentration of the DPC micelles in solution.

When we performed the Bruggemann effective-medium analysis, we found that each DPC micelle entraps a hydration shell composed of 310 ± 45 water molecules. Unlike the naïve absorbance-based method used in prior literature [42], which estimate the size of the hydration shell by assuming that the macromolecule's absorption falls to zero at its minimum, this method of estimating the size of the tightly-bound hydration shell requires only the well-founded assumption that the micellar absorption falls to zero at zero frequency [35]. The value of 310 water molecules for the THz-defined hydration shell has similar size to a monolayer on the surface of the DPC micelles. Specifically, if we approximate DPC as a ~ 2 nm diameter sphere, a solvent layer with one molecule deep will contain 350 water molecules. Using the number of water molecules in the hydration shell related to the scaled filling factor and the measured $\epsilon_{\text{wat}}^*(\nu)$ and $\epsilon_{\text{sol}}^*(\nu)$, we employ eq. (5.8) to obtain the dielectric spectra for several hydrated DPC concentrations (Figure 5.5). It should be noted that the hydration number calculated from the effective-medium approximation is lower than those from the GHz dielectric relaxation spectroscopy. This is expected, because through this method we determine the number of hydration water molecules that have strong hydrogen bonds with the DPC surfactant. These water molecules become an integral part of micelles and cannot move easily; instead, they are held in the tightly-bound hydration layer.

Understanding how micellar dynamics and structure are connected to the chemical composition and geometry of the surfactants offers a considerable challenge. Numerous theoretical approaches and simulations [154-156] have been proposed to predict structure – property relationships. Experimental techniques typically obtained by small-angle neutral scattering [157], static and dynamic light scattering [158], and cryogenic transmission electron microscopy [159], which can probe a wide range of length- and time-scales, are needed to fully characterize micelles and correlate the micellar structure to the dynamical behavior, a fundamental prerequisite for developing practical formulations. The techniques measure the radius of gyration and make a link between micellar structure and dynamics. Here, we directly probe the collective dynamics of DPC micelles with THz radiation using the Bruggemann approximation to exclude the contribution from bulk water for several DPC solutions (Figure 5.5). Upon doing so, we found that these spectra are characterized by a rising dielectric loss and a broad maximum of the dielectric dispersion component. For DPC concentrations below 400 mM, the measured dielectric response extracted from the effective-medium approximation is independent from concentration, suggesting that the size of the tightly-bound hydration shell is likewise independent of DPC concentration. This is the first time that the collective dynamics of DPC micelles at the THz region have

been reported. MD simulations, discussed below, indicate that tightly-bound water molecules are those directly hydrogen-bonded to DPC surfactants. They also explain the collective motions of DPC micelles observed in the THz spectrum.

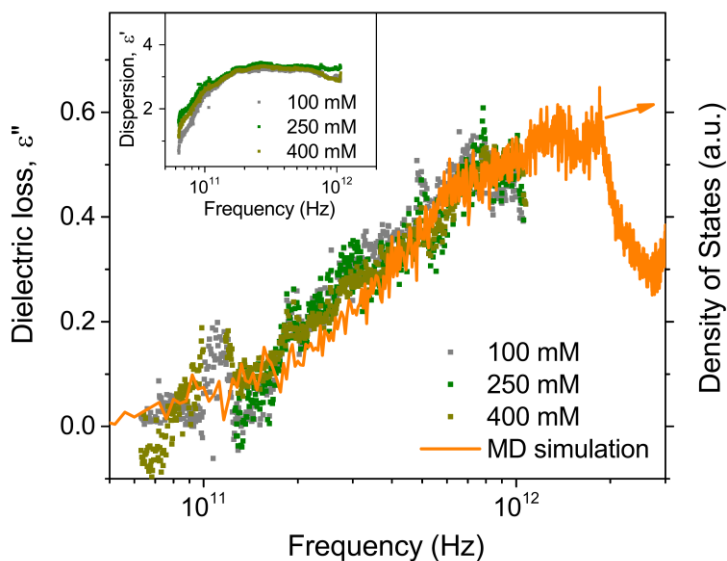


Figure 5.5: Dielectric loss, $\epsilon''(\nu)$, and dispersion, $\epsilon'(\nu)$, (**inset**) spectra of micelles in several DPC solutions at 25°C in the THz frequency range from 60 GHz to 1.2 THz provide insight into the collective motions of micelles using the Bruggemann effective-medium approximation. From the effective-medium approximation, it is found that 310 water molecules in the hydration shell around DPC no more behave as bulk water. The DoS analysis (orange line) from MD simulations was run on the DPC surfactants in the micelle only (no waters are contributing) [75].

5.3.3. Molecular dynamics simulations

To gain insight into the experimental observation of the dynamics and structure of hydration waters, the motion of head groups of DPC surfactants as well as the collective motions of micelles, we have carried out MD simulations for hydrated DPC micelles, for comparison to low concentration dielectric spectra. The combination of MD simulations with GHz to THz spectroscopy provides a microscopic picture of the coupled micelle-solvent dynamics associated with micellar aggregates. The solvation shells of DPC micelles were first analyzed by calculating the solvent radial density functions (Figure 5.6). The radial distribution function (RDF) of water's oxygen was calculated around several atoms of DPC: C_{12} , the carbon nearest the head group; the phosphorous atom; and the nitrogen atom (see Figure 5.1).

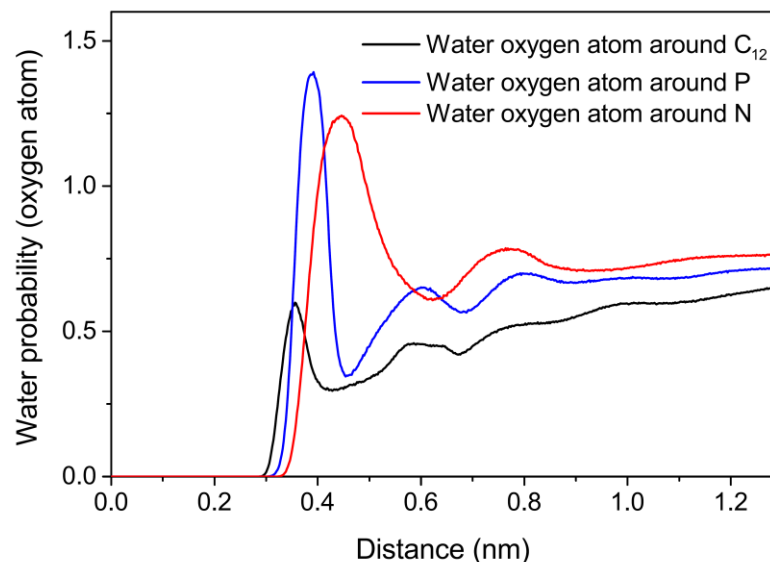


Figure 5.6: The solvent radial distribution functions (water oxygen atom) around C₁₂ (black line), phosphorous (blue line), and nitrogen (red line) atoms of the DPC molecule [75].

The zwitterionic DPC head group has more chemical complexity than most surfactants, but the combined waters within the first peak of these three radial density functions appear to be the first solvation shell (comprised of both loosely-bound and tightly-bound waters, as discussed below). By taking a time average of the number of waters within the first peak of the solvent RDF around DPC head group atoms C₁₂, N, and P, the solvation shell size was calculated to be 995 ± 26 waters. This is in remarkably good agreement with the number of hydration waters (950 ± 45) measured with dielectric spectroscopy at low concentrations of DPC micelle. We also found that by selecting waters within 4 Å of any DPC atom, the solvation shells have similar size, and thus, this definition was used for further analysis of solvation shell dynamics in DPC micellar simulations.

It was observed that the DPC lipid can have multiple head group conformations (Figure 5.7) [155, 160]. These include an extended monomer, in which the DPC molecule remains fairly linear (Figure 5.7a); an intramolecular zwitterionic motif, in which the head group folds over by Coulombic attraction to itself (Figure 5.7b); and a vicinal coupling motif, in which neighboring DPC molecules' cationic and anionic portions are in close proximity (Figure 5.7c). Overall, the surfaces of DPC micelles are rough, having channels that are primarily lined with the cationic amine groups, with some anionic (phosphate) oxygens also being surface-exposed. The surface-exposed atoms are illustrated in Figure 5.8, left panel, with trimethylamines pictured in aqua, and oxygens in red. Figure 5.8, right panel, shows waters within the channels, with the whole micelle surface pictured in dark blue. It can be seen that waters at the surface of the micelle are somewhat confined. Along with Coulombic interactions at the surface, this leads to a slowdown in water dynamics.

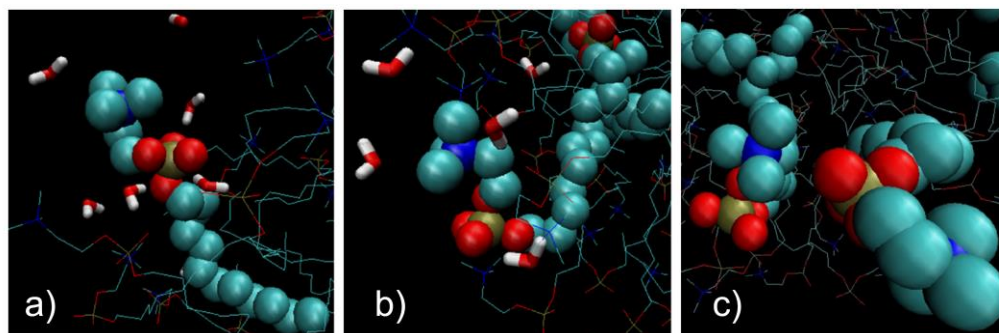


Figure 5.7: MD simulations show different conformational states of a DPC molecule. Solvation motifs: (a) extended monomer (b) intramolecular zwitterionic coupling (c) vicinal zwitterionic coupling [75].

Water molecules in the solvation layer around and within proteins have been found to have drastic reductions in dynamics, on the timescale of 500 ps by dielectric spectroscopy [102]. It is interesting to note that the cationic methylamine groups of DPC are much softer ions than are typically found in protein structures, with an effective radius larger than the Rb^+ cation. As discussed by Abel *et al.*, the methyl groups of the amine fully shield the nitrogen and, consequently, water molecules contact only the methyl groups on the cation [126]. Previous dielectric spectroscopy experiments have shown that larger monocations have a significantly smaller effect on water dynamics than harder, smaller ions such as Na^+ or Li^+ [105]. Water around proteins also experiences the largest slowdown in dynamics within confined or concave spaces [161]. The divots or “canals” seen along the micelle surface are not as confining as protein active sites or interdomain regions. Furthermore, DPC only has hydrogen bond donors, rather than a multitude of hydrogen bond donors and acceptors, as is found in proteins. Therefore, it should not be surprising that no hydration dynamics around the timescale of 500 ps were found in the DPC micelle solvation shell. Rather, the longest dynamics measured in the GHz spectroscopy can be attributed to the motion of head groups of DPC surfactants within the micelle.

The only hydrogen bonding groups on the DPC molecule are the four oxygens around phosphorous, with the phosphate oxygens surrounded by a higher number of waters than the ester oxygens [126]. Waters with hydrogen bonds to surfaces typically result in slower reorientation times [162]. Therefore, solvation shell waters participating in hydrogen bonds with DPC oxygens were evaluated separately. It was found that, on average, there are 297 ± 17 hydrogen-bonded waters throughout the trajectory. This is in excellent agreement with the number of tightly-bound waters (310 ± 45) measured with dielectric spectroscopy at THz frequencies using the effective-medium approximation method.

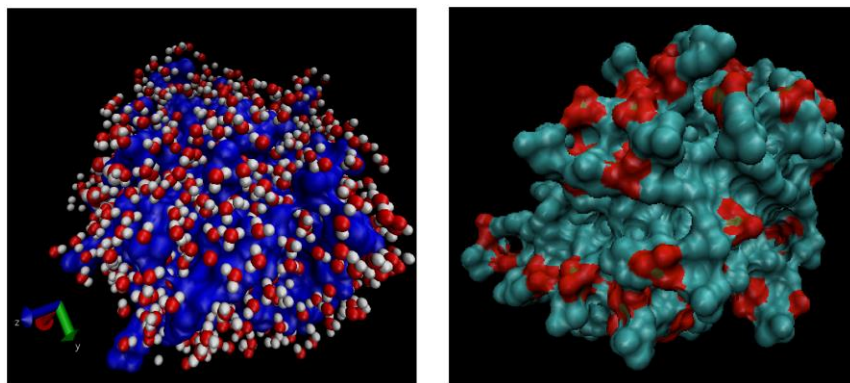


Figure 5.8: DPC micelle surface rendered (left) with alkyl groups (including trimethyl amine moieties) in aqua, oxygen in red, and phosphorous in gold (no waters are shown), (right) in dark blue, with solvation shell waters pictured in red [75].

The 1st Legendre Polynomial (P_1) of the reorientation autocorrelation function (ACF) for tightly-bound water (directly hydrogen bonded to DPC) and the reorientation of waters within the solvation shell that are not hydrogen bonded to DPC (identified as loosely bound waters) are given in Figure 5.9a. According to the extended jump model, water reorientation is slower when it hydrogen bonds to a surface, and this is reflected in the slower decay of the rotational ACF of tightly-bound (hydrogen-bonded) waters [116]. The reorientational lifetimes of tightly- and loosely-bound waters were found by fitting a triexponential decay function. The long-time components of tightly- and loosely-bound waters were calculated to be 115 ± 5 and 42 ± 10 ps, respectively. These values are in good agreement with the dielectric spectroscopy measurements, which found characteristic timescales of 105 ps for tightly-bound waters and 31 ps for loosely-bound waters, respectively.

The relaxational dynamics of DPC molecules within the micelle were characterized with the rotational autocorrelation function (Figure 5.9b). The reorientation timescales of head groups on DPC surfactants were determined by fitting a multi-exponential decay function (red traces, Figure 5.9). As described in our recent paper on cetylpyridinium bromide micelles [155], the short timescale of surfactant reorientation, fit to 79 ps for DPC, likely corresponds to spinning of the surfactant about its axis. Meanwhile, the longer timescale, fit at 591 ps, likely corresponds to head group reorientation or waving along its axis (like seaweed swaying in water). Note that the micelle environment confines surfactant rotation, approximately to a conical section, so that reorientation has a very long component of decay that fits to 4.9 ns. The THz-to-GHz spectroscopy of DPC micelles measures a component at 600 ps, which thus can be assigned to surfactant reorientation. It is interesting to recall that a 600 ps component has also been measured by dielectric

spectroscopy at GHz frequencies for SDS [130] and CTAB [139] micelles. While those spectroscopic peaks have been attributed to the diffuse counterions [130], the rotation of stable ion pairs [138, 151], or the hopping of counterions bound to the charged surface of micelles [152], it is also possible that the dipolar relaxation of surfactants of similar size, shape, and charge distribution within micelles takes place on a characteristic timescale on the order of ~600-1000 ps.

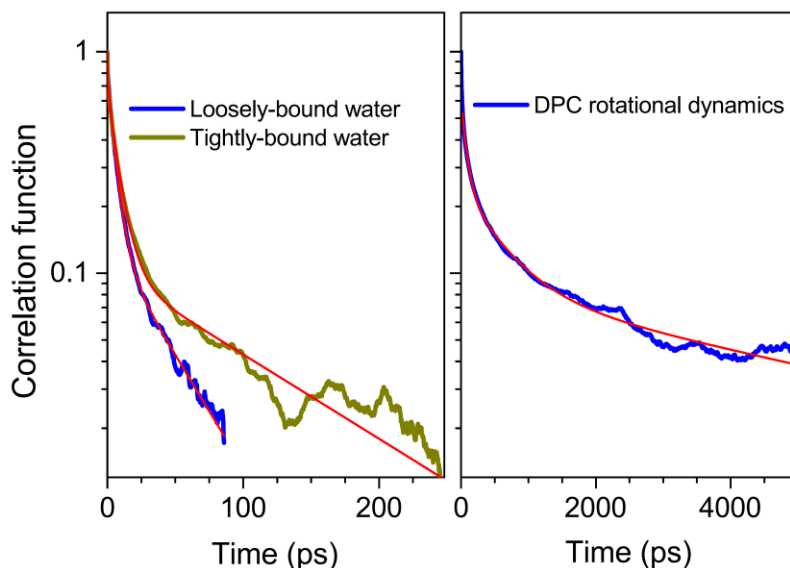


Figure 5.9: Rotational autocorrelation functions, $P_1(t)$ for hydration waters and DPC micelles show multiple-exponential decay behaviors. **(left)** The rotational autocorrelation functions of solvation shell waters hydrogen-bonded to DPC (dark yellow line) and other solvation shell waters (blue line) indicate a difference in the dynamics of tightly- and loosely-bound waters, respectively. **(right)** The rotational autocorrelation function of DPC monomers (blue line) within the micelle explains the dielectric response timescale from dynamics of DPC at 600 ps, arising primarily from the motion of surfactant head groups [75].

The contribution of the collective dynamics of DPC within the micellar environment to the THz spectrum can be seen from MD calculations for the density of states (orange curve, Figure 5.5). The data from MD simulations come solely from the density of states calculation for the micelle itself (not including water). It can be seen that the collective dynamics of surfactant within the DPC micelle make major contributions to the THz spectrum. These motions within a micelle involve bending and undulation of surfactant molecules, including movements of the head group and curving of the alkyl tail [155]. These collective dynamics of micelles have been studied previously using neutron spin-echo spectroscopy [154] and light scattering [163], with surfactants exhibiting breathing modes and worm-like motions [163]. The multiple conformations observed for

the DPC head groups (Figure 5.7) indicate that the dynamics of DPC within the micelle may be driven in part by interactions between the cationic and anionic portions of the head groups. The surfactants can transition between intramolecular and intermolecular (vicinal) zwitterionic coupling, as well as having extended conformations out into the hydration layer. In summary, these multiple conformations are evidence of the structural diversity and dynamic processes of surfactant within the DPC micelle. Future work in DPC simulations is planned to determine how water models influence head group conformational structure and dynamics.

5.4. Conclusion

We have conducted high-precision dielectric spectroscopy of DPC micellar solutions in a wide frequency range from 50 MHz to 1.12 THz to characterize the structure and dynamics of zwitterionic micelles and solvation, and used MD simulations to explain experimental results. The low frequency part indicates that four different relaxational processes occur in DPC aqueous solutions, including the motion of DPC surfactant head groups, which have a reorientation time of ~ 600 ps; hydration waters with times on the order of 30 to 150 ps; and bulk water with a time of 8 ps. There are two types of hydration water molecules in DPC micellar solutions, which are tightly- and loosely-bound waters at the DPC micellar surface. The total amount of hydration water of 950 molecules per micelle has been obtained from the dielectric strengths of bulk water in DPC solutions, which is in excellent agreement with the number of waters in MD simulations found within a distance of 4 Å from the DPC head group atoms. Using the effective-medium approximation for the dielectric measurements at THz frequency, we are able to extract the amount of tightly-bound waters in the first hydration shell of 310 molecules. The observation is in excellent agreement with MD simulations, which indicate ~ 300 waters directly hydrogen bonded to DPC. These water molecules have slower reorientational dynamics than the rest of the solvation shell.

It is interesting to note that, although the phosphatidylcholine head group is comprised of 11 heavy atoms (not H) and two ionic portions, the only hydration waters that are tightly bound are those in direct contact with the oxygen atoms (primarily, the two anionic oxygens). It is known that the dynamics of hydration waters can strongly influence the structure and dynamics of biomolecules, but our ability to predict how biomolecular structures influence water dynamics is quite limited. These results suggest that the confinement effects seen in proteins, which dramatically slow water dynamics, are not present in the rough terrain of the micellar surface. The cationic trimethylammonium groups likewise have a modest effect on water dynamics, being soft ions incapable of hydrogen bonding. Rather, the dominant structural factor affecting water dynamics in the

presence of the DPC micelle is hydrogen bonding atoms. This is useful information for future work, when the effects of lipid membrane composition on water dynamics, and the interplay of hydration dynamics with lipid membrane and membrane protein properties, may be considered.

Finally, at the THz frequency, we have observed the collective vibrational modes within DPC micelles. Simulations indicate that the dominant contribution to the peaks comes from large-scale motions of the lipids within the confining environment of the micelle. These studies represent the first time that a clear peak has been experimentally observed and identified in the dielectric spectroscopy of membrane mimics.

Chapter 6

Insights into Hydration Dynamics and Cooperative Interactions of Glycerol-Water Mixtures

This chapter contains original results submitted for publication:

A. Charkhesht, D. Lou, B. Sindle, N. Q. Vinh. *The Journal of Physical Chemistry B* (2019)

In this chapter, we report relaxation dynamics of glycerol-water mixtures as probed by a megahertz-to-terahertz dielectric spectroscopy in a frequency range from 50 MHz to 0.5 THz at room temperature. The dielectric relaxation spectra reveal several polarization processes at the molecular level with different time constants and dielectric strengths, proving an understanding of the hydrogen-bonding network in the glycerol-water mixtures. We have estimated the hydration effect for molecular interactions as a function of glycerol concentration in solutions using the Debye relaxation model. The experimental results show an existence of a critical concentration of ~7.5 mol % that connects to the number of water molecules in the hydration layer. At higher glycerol concentration, water molecules disperse in the glycerol network, showing four relaxation processes in glycerol-water mixtures. The results reveal critical information of molecular dynamics in solution, providing an understanding of reactivity of co-solvents, glycerol, in aqueous solutions.

6.1. Introduction

Along with water, a variety of co-solvents plays an important role in biological systems [164-168]. The presence of co-solvents changes the behavior of water such as hydrogen-bonding network, dynamics, polar property, and spatial distribution [169]. Co-solvents stabilize the activity of enzymes and native structure of proteins [166], increase the solubility of a nonpolar drug up to several orders of magnitude compared to the aqueous solubility, and enhance the chemical stability of a substance. The study of dynamics of these chemical biomolecules is indispensable to get a comprehensive perception of their conduct in their solutions. Glycerol ($C_3H_8O_3$) is an important co-solvent in this context, which was a subject of numerous studies in molecular dynamics theme [25, 170-173], and

experiment [174-176]. This trihydric alcohol is a colorless, sugar-like, highly viscous alcohol with three hydroxyl groups. The highly flexibility and viscosity of glycerol makes it a decent contestant in glass-forming studies [177]. Likewise, glycerol has been used to preserve proteins because of its cryoprotective properties [178], and stabilize enzymes activities [165, 166]. The ability of forming hydrogen bonds with water makes glycerol-water mixtures fascinating solutions for hydration dynamic mapping [25], enhancing solubility of a poorly water soluble drugs [167], and forming a main co-product of biodiesel and oleochemical production [179, 180]. Thus, a comprehensive understanding the hydration dynamics and cooperative interactions of glycerol-water mixtures provides us the role of glycerol in chemical activities.

The investigation of molecular dynamics in complex liquids is a major challenge among the experimental and theoretical communities. Glycerol and glycerol-water mixtures have been a subject of numerous investigations including molecular dynamics [25, 172, 173], broadband dielectric spectroscopy [174, 177, 181, 182], nuclear magnetic resonance (NMR) [183], infrared [172], and Raman [184] spectroscopy. Although these reports have provided a wide range of techniques to investigate the hydrogen-bonding dynamics of glycerol-water mixtures in different frequency ranges, the hydration dynamic mapping of glycerol in solution is yet to be elucidated. The dielectric relaxation spectroscopy that measures the hydrogen-bond rearrangement dynamics would be a handy tool in order to achieve the understanding of the hydrogen-bonding network in glycerol-water mixtures.

Recent developments in the megahertz-to-terahertz technology provide us a possibility to conduct dielectric response measurements in a large range of time scales to reveal intermolecular interaction of hydrogen-bonding network in the glycerol-water mixtures. We have employed this technique to develop our spectrometer in order to investigate hydration shell dynamics and properties of water molecules interacting with proteins and micelles. The results help us in mapping detailed information related to different molecules [35, 75, 103]. Our developed spectroscopy technique combines important elements of a large spectral range from megahertz to terahertz frequencies and a significant improvement of the signal-to-noise ratio with high power, providing a high-fidelity coverage that no other technique can match [16]. In the present study, we focus on the nature of the hydration dynamics and the associated dynamics of the glycerol in solution at the molecular level. From the complex dielectric response of glycerol-water solutions, we have explored relaxation processes in wide range of concentrations. The hydrogen-bonding related to distinct relaxation times of water molecules in bulk water, hydration layers, and in the glycerol network has been discussed. A critical value of molar percentage (mol %) is considered, and the dynamic structure of different domains based on each region has been designated. The better understanding of hydrogen-bonding characteristics of glycerol-water mixtures is essential to understand the functionality of glycerol molecules as a colligative solute.

6.2. Experimental Methods

6.2.1. Materials

Glycerol solution ($\geq 99.5\%$) with molecular weight of 92.093 g/mol, purchased from Sigma Aldrich (Cat. No. 56-81-5), was employed to prepare glycerol-water mixtures. The mixtures with glycerol content between 5 and 50% volume percentage with an increment of 5 vol % were prepared from the pure glycerol solution and deionized water (resistivity of 18.2 M Ω .cm). Pure glycerol and water were also measured, and the results have been included in our discussion. Table 6.1 Glycerol-water mixtures concentration table shows the glycerol volume percentage (vol %), weight-by-weight ratio (w/w), and a conversion to the molar percentage (x_{glyc}) of our glycerol-water mixtures.

Table 6.1 Glycerol-water mixtures concentration table.

Glycerol volume percentage (vol %)	Weight ratio, w/w	Glycerol molar percentage, x_{glyc} (mol %)
0	0.000	0
5	0.066	1.27
10	0.139	2.65
15	0.221	4.15
20	0.313	5.78
25	0.418	7.56
30	0.537	9.51
35	0.675	11.66
40	0.836	14.05
45	1.026	16.71
50	1.254	19.69

6.2.2. Dielectric Spectroscopy

Dielectric relaxation properties of glycerol-water mixtures at megahertz-to-terahertz frequencies provides insights into the structure and the dynamics of dipolar liquids. The technique is absolutely essential to extract different dynamics of water

molecules in bulk water, hydration layers, glycerol network, and to probe the relaxation process of glycerol. Our spectrometer allows us to study the relaxational (rotational) as well as translational processes of water (bulk, bound-, and slow or confined- water molecules), and glycerol/biomolecules.

The dielectric relaxation spectroscopy of liquid would be the best tool to reveal different components in molecular dynamics of the network. The dielectric spectroscopy of glycerol-water mixtures in a range of 10 μ Hz to 30 GHz at temperature from 148 to 323 K performed by Hayashi et al. [174, 177], and Puzenko et al. [177], and focused on water-rich and glycerol-rich regions [182]. The analyses focused on the dielectric loss in both regions, and used of known phenomenological relations and their superposition for simultaneous fittings. They concluded that the main dielectric relaxation process, the high-frequency “excess wing,” and the dc conductivity in glycerol-water mixtures have the same origin. A critical concentration was extracted for different relaxation processes of molecules in glycerol solutions, resulting from ice nanocrystals or pure water (w-w interactions), pure glycerol (g-g interactions), and glycerol-water (g-w) interactions. However, due to the limitation of the frequency, their studies could not bring up exclusive details about hydration layer dynamics that would be placed in higher frequencies, the “excess wing.” Alternatively, Dashnau et al., performed infrared spectroscopy on glycerol-water mixtures to study hydrogen-bound patterns and cryoprotective properties in various concentrations [172]. They presented how the increasing of glycerol concentration in solution would alter hydrogen-bonding and hydration shell properties. The Fourier transform infrared spectroscopy (FTIR) and molecular dynamics simulations show that CH and OH stretch modes are under influence of glycerol concentrations, altering the interaction of water molecules with glycerol in the hydration shell. Using our large frequency range spanning from megahertz-to-terahertz, we could reveal a picture of relaxation dynamics of water molecules interacting with glycerol within the hydration shell as well as processes related to glycerol molecules.

At the microscopic scale, numerous polarization effects or dielectric mechanisms give rise to the dielectric properties of mixtures. Glycerol/biomolecules and water molecules with permanent dipole moments rotate to follow an alternating electrical field from a radiation source. Each dielectric mechanism has a characteristic frequency. In the megahertz-to-terahertz frequency range, electronic and atomic mechanisms are comparatively weak, and contribute a constant level. Over this region, the dielectric response of aqueous solutions mainly receives contributions from three main regions (a) the rotational motion of solvent (glycerol or biomolecules), i.e., orientational polarization of solvent dipoles; (b) the orientational relaxation of bulk water molecules, i.e., water dipoles; and (c) relaxation process of hydration water in the interfacial region surrounding glycerol/biomolecules, i.e., dipoles of water molecules in hydration layers, or confined water molecules in the solvent network [16, 75, 103, 131].

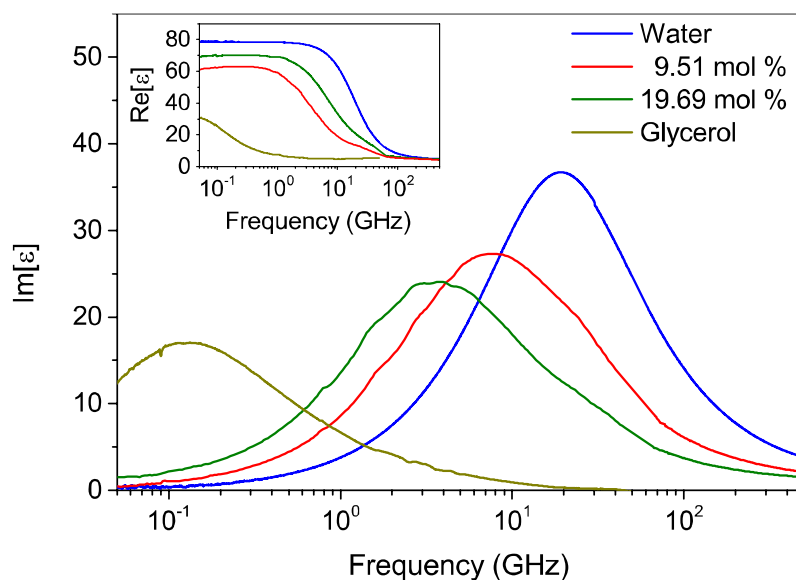


Figure 6.1: Interaction of electromagnetic wave in the megahertz-to-terahertz region with glycerol-water mixtures providing insight into the molecular dynamics over the picosecond to sub-microsecond timescales. The imaginary, $\epsilon''_{\text{sol}}(\nu)$, and the real, $\epsilon'_{\text{sol}}(\nu)$, (in the inset) components of the dielectric response spectra were collected for different concentrations of glycerol in solutions. The maximum of imaginary component centered at ~ 19.2 GHz for pure water moves to lower frequencies for glycerol-water mixtures, and stays at ~ 144.7 MHz for glycerol liquid [Charkhesht A., et al., *The Journal of Physical Chemistry B* (2019) (Submitted)].

To examine the relaxation processes of water and glycerol in solution, complex dielectric measurements of each sample were carried out in a large frequency range from 50 MHz to 0.5 THz at 25 °C. Using an enhanced open-end probe (Agilent 85070E) and a vector network analyzer (Agilent PNA N5225A), we performed analyses in a frequency range from 50 MHz to 50 GHz. The calibration process of this system was performed under three standards including air, pure water, and mercury for short circuit. The complex dielectric response including the real (dielectric dispersion), $\epsilon'_{\text{sol}}(\nu)$, and the imaginary (dielectric loss), $\epsilon''_{\text{sol}}(\nu)$, components was evaluated using Agilent software with accuracy of $\Delta\epsilon/\epsilon = 0.05$. The dielectric response of glycerol-water mixtures at terahertz frequencies has been collected using a gigahertz-to-terahertz spectrometer based on the above vector network analyzer with frequency extenders from Virginia Diodes. The system is capable of simultaneously measuring intensity and phase over a large effective dynamical range [16]. The solutions were kept in a sample cell made of anodized aluminum at 25 °C and controlled with an accuracy of ± 0.02 °C using a Lakeshore 336 temperature controller. The complex dielectric response of glycerol mixtures can be expressed as a function of frequency, ν :

$$\epsilon_{\text{sol}}^*(\nu) = \epsilon'_{\text{sol}}(\nu) + i\epsilon''_{\text{sol}}(\nu). \quad (6.1)$$

Figure 6.1 shows dielectric response spectra of different glycerol concentrations in solutions for both real and imaginary components. The concentration of glycerol in the mixtures is increasing from 0 mol % (pure water) to ~ 20 mol % of glycerol in solution. Dielectric spectra of pure glycerol and pure water are included for reference. When the concentration of glycerol increases, the absorption of the samples decreases dramatically and the maximum of the dielectric loss shifts significantly toward the lower frequency. The decrease in absorption and shift in frequency is expected by comparing the polarity and molecular weight of water and glycerol that affect orientational relaxation of related dipoles [75, 103, 177].

6.3. Results and Discussion

The dielectric properties of aqueous solutions present a complex behavior, originating from different, and/or partially overlapping, polarization mechanisms. In order to determine the contribution of different components in a solution to the dielectric response, the data were analyzed by simultaneously fitting real and imaginary components to relaxation models based on a sum of n individual contributions in the Havriliak-Negami function [185]:

$$\epsilon^*(\nu) = \epsilon_{\infty} + \sum_{j=1}^n \frac{\epsilon_{j-1} - \epsilon_j}{\left(1 + (i2\pi\nu\tau_j)^{1-\alpha_j}\right)^{\beta_j}}. \quad (6.2)$$

The relaxational mode, j , is presented by an individual relaxation strength, $\Delta\epsilon_j = \epsilon_j - \epsilon_{j+1}$, relaxation time, τ_j ($\tau_j > \tau_{j+1}$), and shape parameters, $0 \leq \alpha_j < 1$ and $0 < \beta_j \leq 1$. The basic shape parameters are representatives for the Cole-Davidson relaxation model with $\alpha_j = 0$; $0 < \beta_j \leq 1$, the Cole-Cole with $0 < \alpha_j \leq 1$; $\beta_j = 1$, or the Debye with $\alpha_j = 0$; $\beta_j = 1$. With $j = 1$, $\epsilon_{j-1=0}$ is equivalent to ϵ_s , the static permittivity which can be outlined as $\epsilon_s = \epsilon_{\infty} + \sum_{j=1}^n \Delta\epsilon_j$. With $j = n$, $\epsilon_{j=n} = \epsilon_{\infty}$ indicates of dielectric contributions from modes at frequencies much greater than the experimental range, which reflects inputs from molecular and atomic oscillation dynamics at higher frequencies. It should be mentioned that in the megahertz-to-terahertz frequency range, librational motions and inertial effects do not have a noticeable contribution to the dielectric property and show a constant over the region (ϵ_{∞}).

We have fitted eq. (6.2) concurrently to the measured real, $\epsilon'_{\text{sol}}(\nu)$, and the imaginary components, $\epsilon''_{\text{sol}}(\nu)$, of the dielectric response to attain minimum values of

reduced errors. To examine carefully our data with different relaxation models, Debye-type equations are fully adequate to analytically represent our spectra. We have acquired superposition of four Debye relaxation processes for glycerol-water solutions in the form:

$$\epsilon_{\text{sol}}^*(\nu) = \epsilon_{\infty} + \frac{\epsilon_s - \epsilon_1}{1 + i2\pi\nu\tau_1} + \frac{\epsilon_1 - \epsilon_2}{1 + i2\pi\nu\tau_2} + \frac{\epsilon_2 - \epsilon_3}{1 + i2\pi\nu\tau_3} + \frac{\epsilon_3 - \epsilon_{\infty}}{1 + i2\pi\nu\tau_4} \quad (6.3)$$

where $\Delta\epsilon_1 = \epsilon_s - \epsilon_1$, $\Delta\epsilon_2 = \epsilon_1 - \epsilon_2$, $\Delta\epsilon_3 = \epsilon_2 - \epsilon_3$ and $\Delta\epsilon_4 = \epsilon_3 - \epsilon_{\infty}$ are dielectric strengths of each Debye relaxation process, corresponding to τ_1 , τ_2 , τ_3 , and τ_4 relaxation times ($\tau = 1/(2\pi\nu)$).

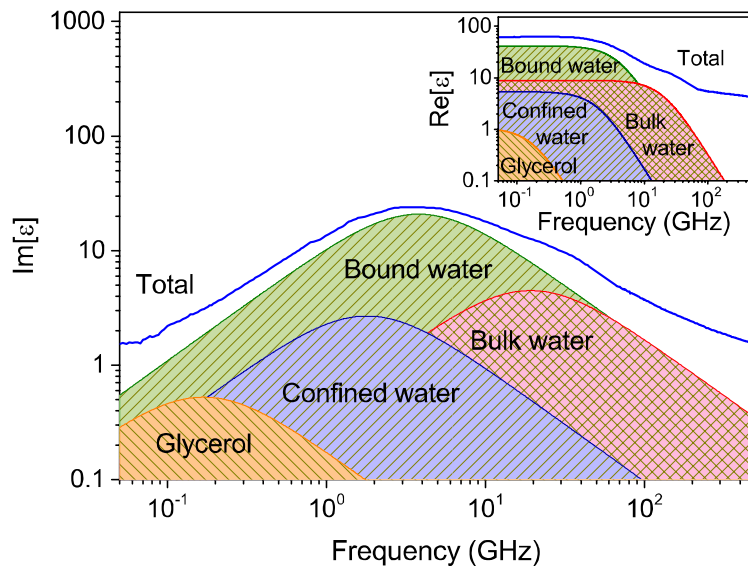


Figure 6.2: Dielectric response of the 19.69 mol % glycerol-water mixture in the frequency range from 50 MHz to 0.5 THz reflecting the complexity of glycerol-water interactions. The imaginary and the real (in the inset) components of the glycerol-water solution have been decomposed in to four relaxational processes with different relaxation time constants [Charkhesht A., et al., *The Journal of Physical Chemistry B* (2019) (Submitted)].

We fit both the dielectric dispersion, $\epsilon'_{\text{sol}}(\nu)$, and loss, $\epsilon''_{\text{sol}}(\nu)$, with a set of free parameters. Parameters in eq. (6.3) were varied concurrently, while the relaxation time for bulk water, $\tau_4 \sim 8.27$ ps [16], ($\nu_4 \sim 19.2$ GHz) and pure glycerol, $\tau_1 \sim 1100$ ps ($\nu_1 \sim 144.7$ MHz, fitted by a one-Debye process for pure glycerol solution) used as initial conditions. A deconvolution of the dielectric spectra has been performed for a glycerol-water solution with molar percentage, x_{glyc} , of 19.69 mol %. The dielectric spectra including the real and the imaginary components (Figure 2) indicates four relaxation

processes centered at 168 ± 18 MHz ($\tau_1 \approx 945 \pm 95$ ps), 1.7 ± 0.2 GHz ($\tau_2 \approx 88 \pm 9$ ps), 3.8 ± 0.5 GHz ($\tau_3 \approx 42 \pm 8$ ps), and 19.2 ± 0.8 GHz ($\tau_4 \approx 8.27 \pm 0.35$ ps).

Corresponding to the four components of the imaginary part, the deconvolution for the real part has been shown in the inset of Figure 6.2. The dielectric constant at higher frequencies, $\epsilon_\infty = 7.06 \pm 0.72$ obtained from the fitting to the four-Debye relaxational model of the glycerol-water mixture is within experimental uncertainty in the literature [16, 59, 107]. We have also obtained dielectric strength values for other processes, $\Delta\epsilon_1 = 1.05 \pm 0.05$, $\Delta\epsilon_2 = 5.36 \pm 0.27$, $\Delta\epsilon_3 = 41.54 \pm 2.10$, and $\Delta\epsilon_4 = 8.95 \pm 0.45$. The relaxation time and the dielectric strength for different concentrations are reported in Figures 6.3 to 6.5. The slowest relaxation time, $\tau_1 \approx 1060 \pm 135$ ps, originates from the dynamics of glycerol in solution. The fastest relaxation time, τ_4 , of 8.27 ps comes from bulk water in the solution. Based on strength and the relaxation time of other processes, one can entitle τ_2 and τ_3 as confined and bound water in the mixtures, respectively. Although probing the dynamics of these water molecules in such mixtures is challenging for years [177], we could clarify the mystery by comparing water dynamics using the Debye relaxation processes in a wide range of glycerol-water concentrations.

6.3.1. Glycerol relaxation

The dielectric parameters of glycerol-water solutions allow us to evaluate relaxational processes of molecules in the mixtures. The relaxation times for four processes are almost constant. The slowest relaxation time, τ_1 , called as the β -relaxation as a function of glycerol concentration is around 1060 ± 135 ps or 150 ± 22 MHz (Figure 6.3, inset). A single glycerol molecule with molar weight of 92.093 g/mol is almost 5 times heavier than water with 18.015 g/mol, a longer relaxation time for glycerol is expected. This value is similar with the relaxation time of glycerol in the pure glycerol solution and within experimental uncertainty of the prior literature values [177, 186, 187].

The dielectric strength for this relaxation process increases with the concentration of glycerol in solution (Figure 6.3, inset). When the concentration of glycerol in solution increases the number of glycerol molecules form glycerol clusters (or bulk liquid glycerol) increase linearly. Thus, we have observed a linear behavior of the dielectric strength for the slowest relaxation process.

The dielectric parameters of the β -relaxation process including the dielectric strength, $\Delta\epsilon_1$, and the relaxation time, τ_1 , provides us an evaluation of the hydrodynamic radius of the glycerol and its electric dipole moment, μ , in their mixture. A simple physical model considers dipoles to be spheres whose rotation in response to the electrical field is opposed by hydrodynamic friction with solvent.

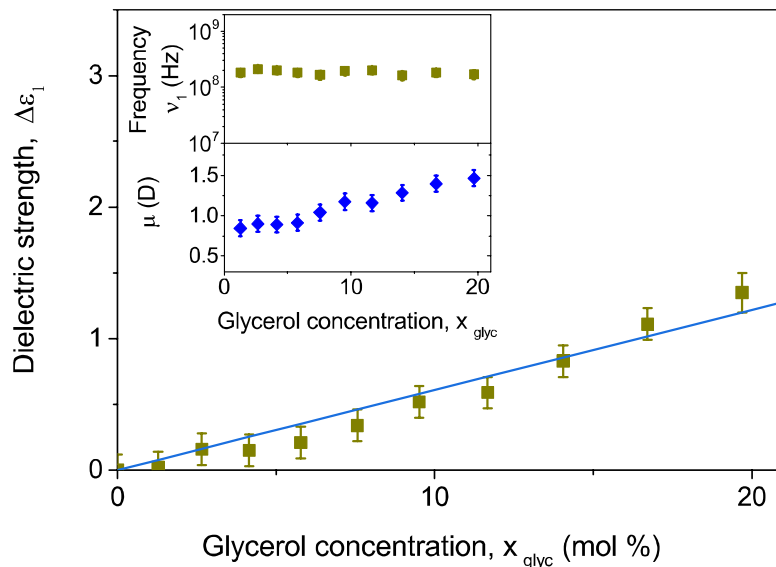


Figure 6.3: Results of dielectric relaxation providing the existence of several relaxation modes in the glycerol-water mixtures. While the relaxation frequency (upper inset) of glycerol, ν_1 , is almost constant with the glycerol concentration, the dielectric strength, $\Delta\epsilon_1$ (ν), of glycerol-glycerol interaction increases with the increasing glycerol concentration. The effective dipole moment values (lower inset) for glycerol in the mixtures have been estimated from the dielectric response [Charkhesht A., et al., *The Journal of Physical Chemistry B* (2019) (Submitted)].

The relaxation time of a spherical molecule a diluted solution with the hydrodynamic radius, R , rotating in a medium of macroscopic viscosity, θ , is given by the Debye equation [188]

$$\tau_1 = \frac{4\pi R^3 \theta}{k_B T} \quad (6.4)$$

where k_B is the Boltzmann constant, T is temperature. At 25 °C the viscosity of an aqueous medium is $0.89 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$ (Pa s), we have obtained the hydrodynamic radius $R = 7.3 \text{ \AA}$ for low glycerol concentration solutions. Note that the size of the spherical dipole is larger than the unit cell dimensions of glycerol crystal in an orthorhombic structure ($a = 7.00 \pm 0.04 \text{ \AA}$, $b = 9.96 \pm 0.05 \text{ \AA}$, $c = 6.29 \pm 0.04 \text{ \AA}$) [189]. This value estimated from the dielectric measurements includes the bound water molecules around a glycerol molecule.

In a liquid constituted by polar molecules, the effective dipole moment of glycerol in solution can be estimated from the dielectric strength. Several approximations for such estimation have been provided in which obtained values for the effective dipole moment should not be overestimated. We employed the same approach as in Ref. [190] to calculate the effective dipole moment of glycerol, μ , by using the Onsager – Oncley model [191]

$$\mu_{\text{eff}}^2 = \frac{2\varepsilon_0 k_B T \Delta\varepsilon_1}{N_A c g_K} \quad (6.5)$$

where N_A is the Avogadro constant, c is the molar concentration (mol/m^3) of glycerol in solution, ε_0 is the permittivity of vacuum, g_K denotes the Kirkwood correlation factor, often assumed to be one in a diluted solution [188, 192]. The obtained values are shown in the lower inset of Figure 6.3. The effective dipole moment of glycerol in mixtures shows a constant of 0.85 D at low glycerol concentration, and it starts to increase with glycerol concentration around 7.5 mol %. At low glycerol concentration, glycerol molecules are well covered by a hydration layer. When the glycerol concentration increases, glycerol molecules tend to form a cluster or a glycerol network (note that the dipole moment of glycerol is 2.56 D), thus, resulting in an increasing of the effective dipole moment of glycerol in mixtures.

6.3.2. Bulk water relaxation and hydration effect

The dielectric spectroscopy provides insights into the dynamics of bulk water, water bound to glycerol, and water confined in the glycerol network. The relaxation time for bulk water, $\tau_4 \approx 8.27$, is independent of glycerol concentration and similar with values reported for pure water at gigahertz frequencies in the literature [16, 59]. However, when adding glycerol to glycerol-water mixtures, the dielectric strength, $\Delta\varepsilon_4$, of the bulk water in the mixtures (Figure 6.4a) reduces significantly. The lowering of the dielectric response with increasing glycerol concentration comes from two main reasons. Firstly, the presence of glycerol in the mixture will reduce the concentration of water in solution, thus lowering the dielectric response of water. Secondly, water molecules form hydrogen-bonding to glycerol. Glycerol, a well-known biomolecule with three OH groups, forms a hydration layer around it in solution. The hydrogen-bonding of water with glycerol increases with increasing glycerol concentration. As a result, the population of water molecules in the hydration layer or around glycerol increases. These water molecules have a different relaxation process and, thus, do not follow the bulk water properties, reducing the dielectric strength of bulk water in the solution.

The average number of water molecules in the hydration layer of glycerol molecule as a function of glycerol concentration provides an important understanding how glycerol functions as a colligative solute. To understand the hydration effect of glycerol in solution, the dielectric strength of the mixtures has been determined under an approximation that all water molecules in the mixture take part in the pure water relaxational mode, so called “*ideal bulk water*.” Figure 6.4a shows the contribution (the solid blue line) of the “*ideal bulk water*” to the dielectric response of glycerol-water mixtures as a function of glycerol concentration. However, the dielectric response of bulk water in the mixtures, $\Delta\varepsilon_4$, aka “*experimental bulk water*” is lower, meaning that not all water molecules in the mixtures

participate in the bulk water, τ_4 , relaxational process. The difference of the dielectric response between the ideal bulk water and the experimental bulk water directly associates with the number of the water molecules missing in bulk water pool. These missing water molecules have hydrogen-bonding to glycerol molecules and relax with different characteristics.

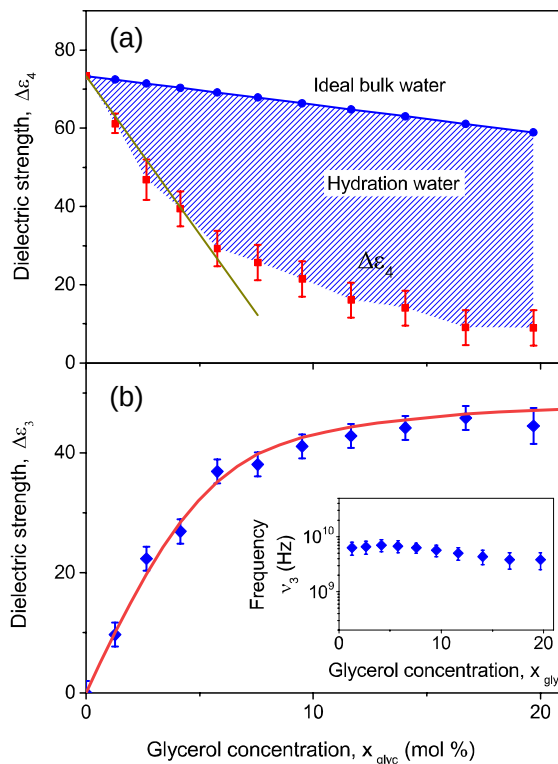


Figure 6.4: Dielectric spectra of glycerol mixtures revealing the number of water molecules affected by the presence of glycerol. (a) The dielectric strength of bulk water in glycerol, $\Delta\epsilon_4$ (ν) decreases significantly the increasing glycerol concentration. The solid line (blue) represents the dielectric strength of the “ideal bulk water” extracted with an assumption that all water molecules in the mixtures behave as pure water, and relax with the time constant of 8.27 ps. A straight line at the low concentration region is a guide for eye. (b) Amplitude of the dielectric property of the bound water in glycerol-water mixtures increases with increasing of glycerol concentration. The solid line in red color is a guide for eye. In the lower inset, the relaxation frequencies of bound water in the hydration layer are almost constant with glycerol concentration [Charkhesht A., et al., *The Journal of Physical Chemistry B* (2019) (Submitted)].

The missing water molecules relax into two different processes, showing from our Debye-relaxation analysis. Specifically, two relaxation processes with the frequency of 4.5 ± 1.5 GHz (Figure 6.4b, inset) and 1.87 ± 0.22 GHz (Figure 6.5, inset), corresponding to the time constants of 35 ± 8 ps and 85 ± 9 ps, respectively. These time constants are longer

than those of the pure water of 8.27 ps. While the former process with the time constant, τ_3 , can be related to bound water molecules around glycerol, the latter with the time constant τ_2 can be assigned to the water molecules staying in the glycerol network. The hydrogen-bonding of water to glycerol is stronger than those in the pure water, thus, the relaxation time constants are longer.

The bound water molecules the time constant $\tau_3 = 35 \pm 8$ ps form the hydration layer which is heterogeneous at the molecular level. The amount of bound water molecules that do not take part in the bulk water rotational process in the solution can be estimated from the dielectric strength of the process, $\Delta\epsilon_3$. Thus, the number of water molecules in the hydration layer of glycerol as a function of glycerol concentration, c , is given [75, 102, 105, 108]:

$$N_{\text{hyd}}(c) = \frac{c_w - \frac{\Delta\epsilon_3}{\Delta\epsilon_{\text{pure}}} c_{\text{pure}}}{c} \quad (6.6)$$

where c_w is the molar concentration of water in the solution, and $c_{\text{pure}} = 55.35$ M is the molarity and $\Delta\epsilon_{\text{pure}} = 73.25$ is the dielectric strength of pure water at 25 °C [16, 55].

The dielectric strength of bound water molecules varies non-linearly with the glycerol concentration. At low glycerol concentration ($0 < x_{\text{glyc}} < 10$ mol %), the dielectric strength increases linearly with glycerol concentration. When glycerol concentration is high ($x_{\text{glyc}} > 10$ mol %), the dielectric strength shows a saturation behavior. Under the low glycerol concentration condition, we have estimated the number of water molecules hidden in the hydration layer around glycerol molecule. Our analyses show that a number of water molecules of 5.58 stays around one glycerol in the low-concentration regime. The average of water molecules in the hydration layer of glycerol is relatively constant in the low glycerol concentration. This value is in agreement with other reports in the literature [172, 193]. At low concentration of glycerol, the mixture is dilute and glycerol molecules spread uniformly in the solution, we can obtain the maximum number of water molecules in the hydration layer. Thus, in the first approximation, the number of water molecules in the glycerol hydration layer is independent of the glycerol concentration. When the glycerol concentration increases to a certain value, hydration layers start to overlap, glycerol molecules aggregate into glycerol clusters, resulting in a decrease of hydration number. The dielectric response of bound water shows a saturation behavior at the glycerol concentration of ~ 7.5 mol %. A similar observation for the hydration aggregation has been reported in dielectric property of bovine serum albumin [103], lysozyme proteins [35, 102], and micelles [75] in a wide range of solute concentrations.

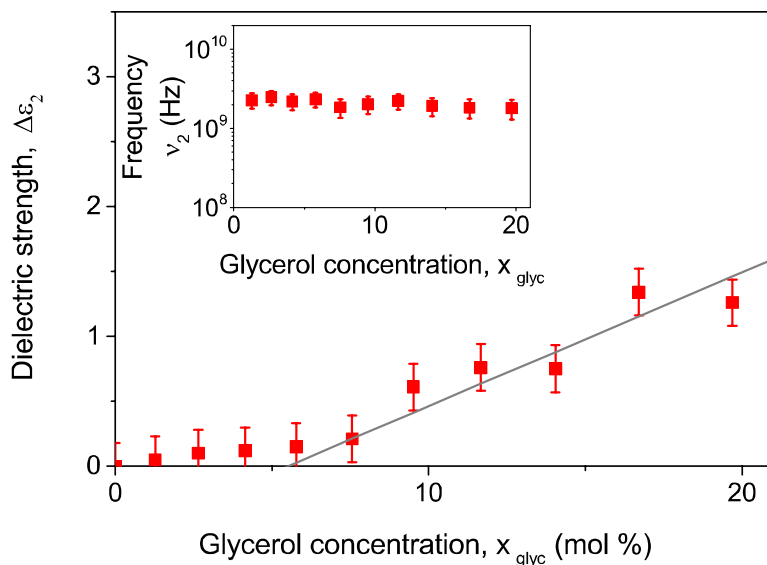


Figure 6.5 A slow dynamics of water in the glycerol network indicating in the dielectric property of glycerol-water mixtures. Amplitude of the dielectric property of confined water molecules in mixtures shows an onset at ~ 7.5 mol %. After the critical concentration, the dielectric strength increases linearly with increasing of glycerol concentration. A solid line is a guide for eye. In the inset, the relaxation frequencies of confined water in the glycerol network are typically constant with glycerol concentration [Charkhesht A., et al., *The Journal of Physical Chemistry B* (2019) (Submitted)].

6.3.3. Confined water in glycerol network

Water and glycerol molecules are well associated in high glycerol concentration mixtures. Dielectric spectra of glycerol-rich mixtures suggested that water cooperative domains would not exist, because water molecules are dispersed well in the mixtures [177, 182]. A long relaxation process, $\tau_2 = 85 \pm 9$ ps, corresponding to the frequency of 1.87 ± 0.22 GHz has been observed in our analysis of the dielectric response (Figure 6.2). The relaxation frequency for this process is almost constant with the glycerol concentration (Figure 6.5, inset). Water molecules having hydrogen-bonding only with glycerol molecules are identified as confined water in the glycerol network, thus, their relaxation time are expected longer than those of water molecules in the hydration layer. The dielectric strength for this process as a function of glycerol concentration shows an onset at 7.5 mol % (Figure 6.5). At low glycerol concentration, the dielectric strength of the confined water is almost negligible. When the concentration of glycerol increases, the dielectric strength for these water molecules starts to increase. This value coincides with the observation of the saturation value of the dielectric strength of bound water in Figure 6.4b, and the increasing of the effective dipole moment of glycerol in mixtures (Figure 6.3,

lower inset). We have obtained here the dynamics of confined water in the glycerol network.

6.4. Conclusion

We have performed the dielectric spectroscopy of glycerol-water mixtures in a wide frequency range from megahertz-to-terahertz region to inspect the systematic transition from pure water towards pure glycerol. The dielectric response of glycerol-water mixtures indicates that four different relaxation processes including the rotation motion of glycerol molecules with the reorientation time of ~ 1100 ps, confined water in the glycerol network with the time constant of 85 ps, bound water in the hydration layer with the time constants of 35 ps, and bulk water with the time constant of 8 ps. Water molecules in the hydration layer and in the glycerol network relax with longer time constants than those of bulk water in solutions.

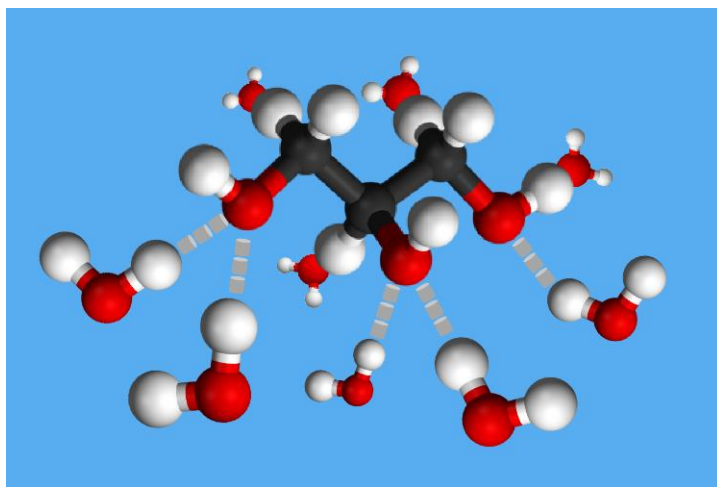


Figure 6.6: Schematic representation of glycerol-water mixtures. This picture shows how water molecules are interacting with glycerol molecules in solution [Charkhesht A., et al., *The Journal of Physical Chemistry B* (2019) (Submitted)].

While the dielectric strength for bound water saturates, the dielectric amplitude for confined water shows an onset at a critical glycerol concentration of ~ 7.5 mol %. In the low glycerol concentration regime, the average of water molecules of 5.58 in the hydration layer of glycerol is relatively constant. In higher glycerol concentration solutions, the hydration shells are merged, the dielectric response for bound water show a saturation behavior, and the dielectric response for confined water increases with glycerol

concentration. The results provide an extending understanding of reactivity of co-solvents, in this case glycerol in aqueous solutions.

Summary

We have discussed in detail the terahertz frequency domain spectroscopy system that we have designed and developed in order to study the dynamical properties of biomolecules in liquid water. Our curiosity to learn more details about these dynamics knows no limits. Therefore, we have been able to extend the frequency of the terahertz band toward the megahertz region to map a wide timescale of relaxation responses of biomolecule. This timescale includes the fast picosecond processes as collective vibrations to slow microsecond processes like rotations. By using the open-end probe and the TFDs systems, we were able to measure the absorption coefficient and refractive index of various solutions in water as buffer from 50 MHz up to 1.12 THz. Thus, we have been able to calculate complex dielectric responses of biomolecules and different types of water molecules around them.

We have chosen three well-known proteins and biomolecules on which to test responses in the frequency range. As we advanced toward determining the molecular dynamics of biomolecules and water molecules, we were able to formulate an explanation for hydration water properties around biomolecules. The existence of hydration shell along with number of water molecules, in different distance and coulombic potential, interacting with biomolecules have been deliberated by MHz-to-GHz dielectric response measurements and Debye model.

In addition to biomolecules and water dynamics, we were able to track down, exclusively, the collective vibration modes of proteins and number of tight water molecules in the hydration shell. The effective medium theory, Bruggeman approximation, was also beneficial, as well as our benchmark terahertz frequency domain spectrometer in the interpretation of dielectric responses and carrying out of measurements.

Appendix A

Soft Phonon Mode Dynamics in Aurivillius Type Structures

This chapter was adapted with only minor changes from the manuscript:

D. Maurya, A. Charkhesht, S. K. Nayak, F-C. Sun, D. George, A. Pramanick, M-G. Kang, H-C. Song, M. M. Alexander, D. Lou, G. A. Khodaparast, S. P. Alpay, N. Q. Vinh, and S. Priya. (2017). Soft phonon mode dynamics in Aurivillius-type structures. *Physical Review B*, 96(13), 134114.

In this part, we step away from biophysics and discuss some other aspects of terahertz science. We report the dynamics of soft phonon modes and their role towards the various structural transformations in Aurivillius materials by employing terahertz frequency-domain spectroscopy, atomic pair distribution function analysis, and first-principles calculations. We have chosen $\text{Bi}_4\text{Ti}_3\text{O}_{12}$ as a model system and identified soft phonon modes associated with the paraelectric tetragonal to the ferroelectric monoclinic transition. Three soft phonon modes have been discovered which exhibit a strong temperature dependence. We have determined that the anharmonicity in Bi–O bonds plays a significant role in phonon softening and that Bi cations play an important role in the emergence of ferroelectricity.

A.1. Introduction

The knowledge of soft phonon mode properties is crucial for understanding the origin of lattice instabilities and structural phase transitions in bismuth layered ferroelectrics (Aurivillius-type structures represented as $[\text{Bi}_2\text{O}_2][\text{A}_{m-1}\text{B}_m\text{O}_{3m+1}]$, where A = Bi, and B=Ti, for $\text{Bi}_4\text{Ti}_3\text{O}_{12}$). Typically, ferroelectric-paraelectric phase transitions in these materials occur with the heavily damped phonons in the terahertz (THz) frequencies [194, 195]. Additionally, there could be subtle structural distortions below Curie temperature (T_c), which are often difficult to correlate with phonon dynamics. Since structural changes drive many material properties, a fundamental understanding of dynamics of these phonon modes is critical for designing high performance ferroelectric materials and devices [196]. The number of phonon modes are defined by the nature of changes in the symmetry during the transitions. The phase transitions involving more than one soft phonon modes [197]

and corresponding order parameters, may induce structural transformations at temperatures below T_c . However, the condensation of more than one phonon modes at a single transition is quite unusual [198].

Here, we employed $\text{Bi}_4\text{Ti}_3\text{O}_{12}$ (BiT) as a model system to understand phonon modes related to phase transitions in Aurivillius materials. The ferroelectric members in these families have potential for high temperature sensors and fatigue-free ferroelectric memory devices etc. [199]. Furthermore, these layered materials exhibit anisotropic and very low thermal conductivity due to effective phonon scattering [200]. The structure of BiT consists of perovskite-like block $(\text{Bi}_2\text{Ti}_3\text{O}_{10})^{2-}$ interleaved with fluorite like $(\text{Bi}_2\text{O}_2)^{2+}$ layers perpendicular to pseudo-tetragonal c -axis [201] which results in relatively higher polarization [202]. In terms of phase transformation characteristics, BiT undergoes a ferroelectric phase transformation from the high temperature tetragonal paraelectric phase to a lower temperature polar phase [203]. This phase transition involves displacement of Bi atoms within the perovskite layers and the rotation of the TiO_6 octahedra [204].

Using a sensitive and high resolution THz frequency-domain spectroscopy, we have experimentally discovered the so - far elusive - three phonon modes in the BiT system. These phonon modes, not reported earlier, are expected to have important implications towards the symmetry breaking from the high temperature tetragonal to the low temperature monoclinic phase as well as structural transformations below T_c . We have further employed atomic pair distribution function (PDF) analysis to correlate the dynamics of Bi ions with the observed phonon dynamics. These results are complemented by first principles based phonon studies, which describe the THz spectroscopy and identify that the main contribution to the atom-projected phonon density of states (DOS) comes from the Bi atoms.

Much effort has been devoted to understand structural changes with respect to temperature in Aurivillius ferroelectric materials. Theoretical [198] and experimental [205] studies have reported possible triggered phase transitions from low temperature polar monoclinic phase to high temperature tetragonal phase of BiT. The low temperature ferroelectric monoclinic phase of BiT requires condensation of at least three different symmetry breaking modes, which have hitherto not been observed experimentally [198]. An observation of the temperature dependence of the lowest frequency polar phonon mode (denoted as a soft phonon mode), using Raman scattering is not very convincing, because, the intensity of the soft phonon mode decreases rapidly with increasing temperature [197]. On the other hand, THz frequency-domain technique, used in this study provides direct measurement of optical phonons providing opportunity to understand the basic nature of transformations.

Prior studies have investigated the dynamics of the ferroelectric transition in Bi-layered ferroelectric materials using THz time-domain spectroscopy [197, 206], where only one optical soft mode was observed in the ferroelectric phase of the BiT material [197], which

was underdamped above the phase transition temperature (T_c) due to the change of selection rules in the paraelectric phase. However, using our high resolution and large dynamic range THz frequency-domain spectroscopy [16], we have observed multiple optical modes which could explain the various structural transformations leading to the ferroelectric phase in BiT and BiT-like layered materials. We further employed atomic pair distribution function (PDF) analysis and the first-principles calculations to provide the fundamental understanding of phonon dynamics in layered ferroelectrics.

A.2. Experimental Details

The THz experiments were performed on highly textured (00l) oriented BiT ceramics. To confirm phase formation and texture, the X-ray powder diffraction (XRD) spectra were recorded at room temperature by using a Philips Xpert Pro X-ray diffractometer (Almelo, The Netherlands), as shown in Figure A.1.

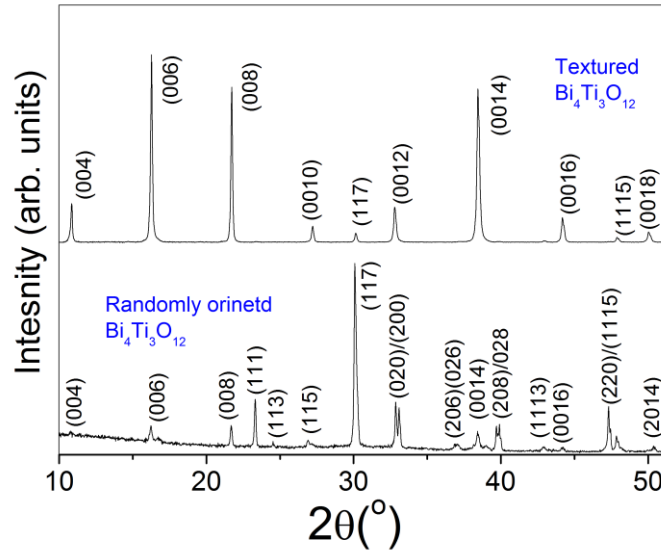


Figure A.1: XRD spectra recorded at RT for textured and randomly oriented BiT ceramics. Please note the change in the intensity of textured BiT ceramics indicating the high degree of the crystallographic orientation along the c-axis [207].

For PDF analysis, high resolution powder X-ray diffraction data was recorded using beamline 11-BM at Argonne National Laboratory. The surface morphology of sintered samples was observed using a LEO Zeiss 1550 (Zeiss, Munich, Germany) scanning electron microscope. Transmission electron microscopy was performed using the

FEI Titan 300 electron microscope. The THz frequency-domain spectrometer employs a commercial Vector Network Analyzer from Agilent, the N5225A PNA which covered the frequency range from 10 MHz to 50 GHz, and THz frequency extenders as well as matched harmonic detectors developed by Virginia Diodes, Inc. with frequency range from 60 GHz to 1.12 THz. The dynamic range of the instrument reaches 10^{13} with a spectral resolution of less than 100 Hz.

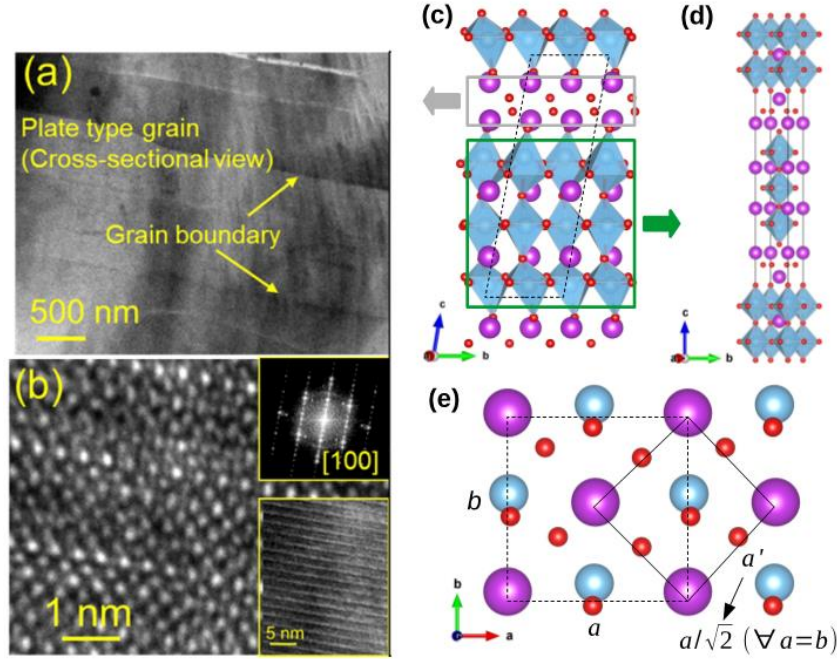


Figure A.2: (a) Bright field cross-section TEM image of plate type grains in BiT indicates that the thickness is in the range of 200–500 nm. (b) The HR-TEM lattice fringe images of BiT ceramics observed from zone axis [100] indicate the stacking of the pseudo-perovskite and $(\text{Bi}_2\text{O}_2)^{2+}$ layers. The lower inset of (b) shows the corresponding low magnification image. Note that images of Bi_2O_2 layers in the HR-TEM image are collected with the electron beam parallel to the [100] zone axis. The upper inset of (b) depicts the corresponding FFT patterns indicating [100] zone axis. Low and high temperature phases of the relaxed BiT structures are shown in (c) and (d), respectively. Bi is denoted by large purple spheres, O by small red spheres. Ti ions stay at the center of the light blue octahedral surrounded by six O atoms. (e) A suggested transformation path from monoclinic to tetragonal symmetries. This transition is associated with the opposite movement of the fluorite- and perovskite-like layers, indicated by gray and green arrows shown in (c), respectively [207].

A.3. Results and Discussion

Figure A.2a shows a bright field cross-section TEM image of textured BiT samples. The cross-section morphology indicates that the plate-type BiT grains are stacked along the thickness of the sample confirming the textured microstructure of the BiT. From these images the size of the plate-type grains is in the range of 5–15 μm . The thickness of these plate type grains was found to be in the range of 200–500 nm (Figure A.2a). The stacking of the pseudo-perovskite and $(\text{Bi}_2\text{O}_2)^{2+}$ layers was clearly observed in the HR-TEM lattice fringes from [100] zone axis, as shown in Figure A.2b. The upper inset in Figure A.2b shows the FFT pattern indicating [100] zone axis, whereas, the lower inset indicates a TEM image with low magnification, revealing layered structure. Due to the two-fold in-plane symmetry, the distinctive stacking of the pseudo-perovskite and $(\text{Bi}_2\text{O}_2)^{2+}$ layers was not observed from [001] zone axis. The schematic of BiT layered structure at low temperature phase is provided in Figure A.2c.

The high dynamic range and high resolution of our THz frequency-domain spectroscopy allows us to observe the lowest-frequency polar phonon modes or soft phonon modes. The refractive index, $n(\nu)$, and absorption coefficient, $\alpha(\nu)$, of BiT samples have been determined through THz measurements, as shown in Figure A.2a and b, respectively for several temperatures from room temperature to near T_c at 600°C. From the absorption and refractive index measurements, we have defined the complex dielectric response of the sample. The frequency-dependent complex dielectric response, $\epsilon^*(\nu) = \epsilon'(\nu) - i\epsilon''(\nu)$, is related to the complex refractive index, $n^*(\nu) = n(\nu) - i\kappa(\nu)$, through the relations:

$$\begin{aligned}\epsilon'_{\text{sol}}(\nu) &= n^2(\nu) - \kappa^2(\nu) = n^2(\nu) - (c\alpha(\nu)/4\pi\nu)^2, \\ \epsilon''_{\text{sol}}(\nu) &= 2n(\nu) \cdot \kappa(\nu) = 2n(\nu)c\alpha(\nu)/4\pi\nu,\end{aligned}\tag{A.1}$$

where ν is the frequency of the THz radiation. The real part, $n(\nu)$, is the refractive index and the imaginary part, $\kappa(\nu)$, is the extinction coefficient and indicates the attenuation when the radiation propagates through the material. The extinction coefficient, $\kappa(\nu)$, is related to the absorption coefficient through a relation: $\alpha(\nu) = \frac{4\pi \cdot \nu \cdot \kappa(\nu)}{c}$, where c is the speed of light.

Accounting these relationships, we have obtained the complex dielectric response of the BiT sample including the dielectric loss, $\epsilon''(\nu)$ and permittivity $\epsilon'(\nu)$ as a function of THz frequency at various temperatures up to the Curie temperature of the material (Figure 0.3c and Figure 0.3d), respectively. Unlike the previous reports where only one mode was reported at 0.83 THz [197, 199], we have observed three phonon modes at 0.68 THz (22.68 cm^{-1}), 0.86 THz (28.69 cm^{-1}) and 0.96 THz (32.02 cm^{-1}) at room temperature for the 30 μm BiT sample. The observations were reproducible (3 different samples) and the temperature cycling did not noticeably affect the observed phonon modes. A strong temperature dependence of these phonon modes and the corresponding phonon mode frequencies decreasing toward zero near the phase transition temperature, T_c , appear to

suggest their soft mode behavior. The theoretical calculations further confirm soft nature of the phonon modes. Upon heating, in addition to mode shifting, the full width at half maximum (FWHM) of the absorption peak also increases with temperature.

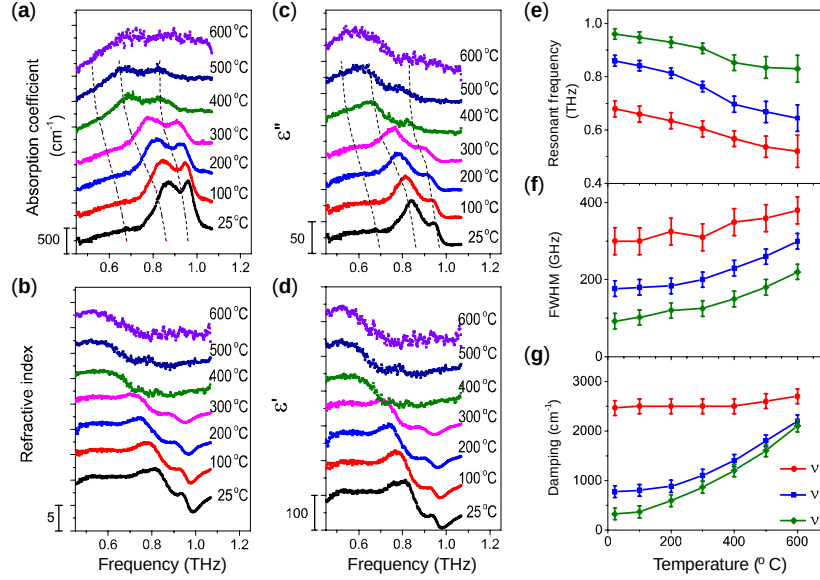


Figure 0.3: The terahertz (a) absorption and (b) refractive index of the *c*-oriented textured polycrystalline BiT ceramic material were recorded at various temperatures. Complex terahertz dielectric response including (c) the dielectric loss and (d) the permittivity at different temperatures calculated from their absorption and refractive index provides insight into the structural dynamics of the BiT material. Employing the three-damped oscillator model, we extracted values for optical phonons (e) soft phonon frequencies ν_1 , ν_2 , and ν_3 , (f) FWHM and (g) phonon damping factors γ_1 , γ_2 , γ_3 . The curves are shifted for clarity in panels (a-d) and the dashed lines are guide for the eye [207].

To gain better insight into the damping process of the three soft phonon modes, we have fitted the complex dielectric response obtained from our THz frequency-domain spectroscopy at various temperatures. For this, we employed a function containing a sum of three damped Lorentz oscillators describing the optical phonons of the ferroelectric materials [208]:

$$\varepsilon^*(\nu) = \varepsilon_\infty + \sum_{j=1}^3 \frac{A_j / (2\pi)^2}{\nu_j^2 - \nu^2 + i\nu(\gamma_j)} \quad (\text{A.2})$$

where $A_j/(2\pi)^2$, ν_j , and γ_j are, respectively, the spectral amplitude of the j damped resonances, its frequency, and its damping coefficient, ε_∞ describes contributions to the dielectric function from modes at frequencies much greater than our experimental range. The parameters of three soft-phonon modes as function of sample temperature are summarized in Figure 0.3e, f, and g. The resonant frequencies of the three soft phonon modes (ν_1 , ν_2 , and ν_3) decreases with increasing sample temperature. The damping of these modes increases with the sample temperature. While the damping and FWHM values for the ν_1 phonon mode slightly changes with the sample temperature, these parameters for the ν_2 , and ν_3 modes exhibit a strong increase with increasing temperature, which suggests a finite coupling between these modes. The results provide an evidence of the soft nature of these phonon modes.

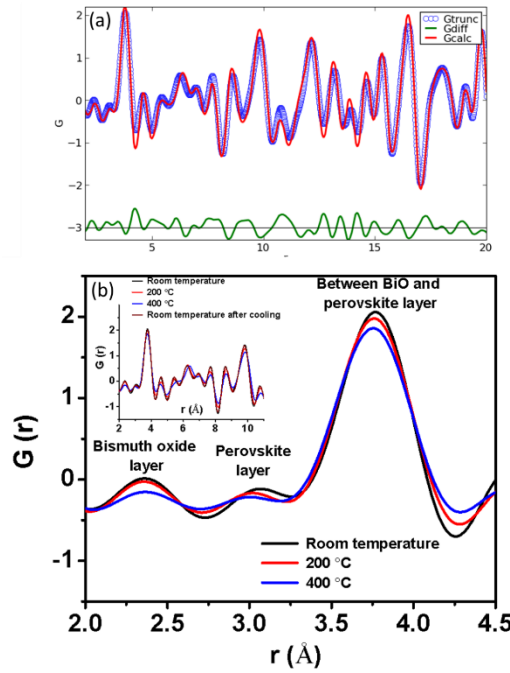


Figure A.4: (a) The fit obtained using PDFGUI for B2cb structure in BIT. (b) Peaks indicate the closest neighbor Bi–O bonds. The Bi–O bonds show significant disordered structure at higher temperatures for both bismuth oxide and the perovskite layers. The inset of Fig. A.4(b) show the pair distribution functions, $G(r)$, measured under different conditions, providing a relation between the dynamics of Bi ions with phonon dynamics. The calculated pattern for the B2cb structure (high temperature orthorhombic phase) is shown with dotted line [207].

The pronounced softening of the ν_2 and ν_3 modes with increasing temperature can be understood as a result of impending ferroelectric-to-paraelectric phase transition as the sample temperature approaches $T_c \sim 700$ °C. The anomalies below T_c could be observed

from the THz spectra, as shown in Figure 0.3. A discontinuity in the temperature dependence and a sharp increase in FWHM for all these modes occur at $T > 300$ °C. The damping of these phonon modes increases significantly when the sample temperature reaches near to T_c . In order to understand the possible structural distortions, which might explain anomalies observed below T_c , an atomic PDF analysis was performed. The PDF measurements obtained from a total scattering XRD pattern via a Fourier transform provides us an approach to study the local structure of materials. Because the total scattering pattern is composed of Bragg as well as diffuse scattering contributions, the information contains local, medium range and long range structure information. The high energy XRD results were corrected for the sample absorption, background, Compton scattering, and incident flux. The intensities were normalized and reduced to the structure factor $S(Q)$ (where Q is the diffraction wave vector), which was Fourier transformed to the corresponding PDFs using PDFgetX [209], $G(r)$. The $G(r)$ gives the probability of finding a pair of atoms at a distance r [210]:

$$G(r) = \frac{2}{\pi} \int_0^\infty Q[S(Q) - 1] \sin(Qr) dQ. \quad (\text{A.3})$$

Having the experimental PDF, one usually wants to determine local structural changes. The PDF results were fitted with B2cb structure having lattice parameters $a = 5.448$ Å, $b = 5.411$ Å, $c = 32.83$ Å, as shown in Figure A.4a. The atomic positions were same as given by Rae et al. [211]. The peaks for the nearest neighbors are highlighted in Figure A.4b. The inset of Figure A.4b shows the experimental PDF, $G(r)$, for the BiT at different conditions. One can clearly see the broadening of the peaks related to the bismuth oxide layer and perovskite layer at 400 °C (Figure A.4b). The broadening of these peaks indicates increasing disorder in bismuth layers. Most notably, the peak related to the perovskite layer is not just broadened, but also, became asymmetric indicating increased anharmonicity of the Bi–O bonds. We have determined that the anharmonicity of these bonds plays a significant role in shifting of the soft phonon modes, and could possibly be the origin of anomalies observed in THz spectra below T_c .

In order to obtain further insights into the experimentally observed phonon dynamics and bond anharmonicity, phonon studies were performed with first principles density functional theory (DFT) [212, 213]. The generalized gradient approximation (GGA) [214] was used as the exchange-correlation functional together with the projector-augmented wave method [215] as implemented in the Vienna *ab initio* Simulation Package (VASP) [216-218]. The primitive cell dimensions for the monoclinic low temperature phase of BiT with space group Pc were found to be $a = 5.49$ Å, $b = 5.53$ Å, $c = 16.88$ Å, and $\alpha_0 = 80.61^\circ$. These values are in good agreement with experimental reports [219] and other first-principles computations [220]. Phonon calculations were performed by linear response method [221] and the frozen phonon method together with Phonopy [222].

Combination of DFT with frozen phonon method provides the platform to analyze lattice dynamics in quasi harmonic approximation with the inter-atomic forces calculated from the state-of-the-art electronic structure methods.

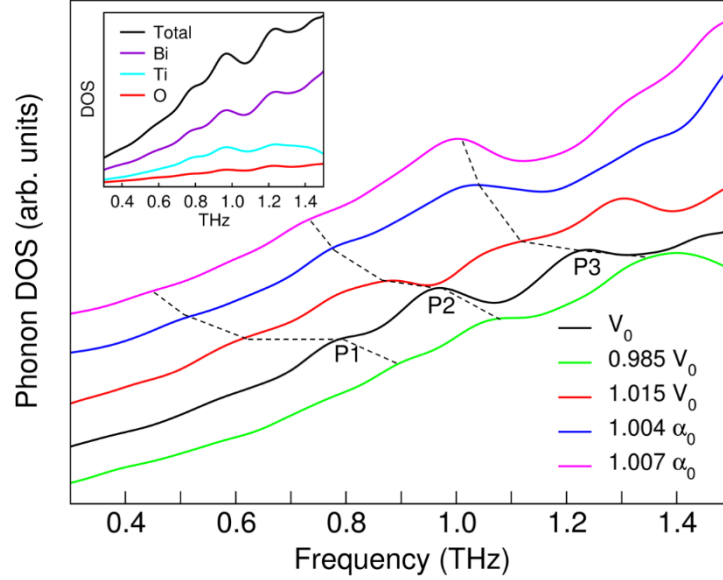


Figure A.5: Phonon density of states (DOS) calculated using frozen phonon method. The phonon DOS for ground state monoclinic structure (V_0) is shown in black solid line. Hydrostatic change in volume by -1.5% ($0.985 V_0$) and +1.5 ($1.015 V_0$) are shown as green and red solid lines, respectively. The DOS for the change in monoclinic angle by 0.04% ($1.004 \alpha_0$) and 0.07% ($1.007 \alpha_0$) from ground state (α_0) are shown in blue and magenta solid lines, respectively. The peaks shift to lower frequencies in all cases due to rearrangement of atomic positions upon relaxation. The dashed lines are guide to the eye. The inset shows atomic contribution to the total phonon DOS, suggesting that a major contribution to phonons in the low frequency range is due to the Bi atoms. The DOS is shifted for clarity [207].

The phonon density of states (DOS) is shown as a black solid line in Figure A.5. We identify three peaks P1, P2 and P3 in the range 0–1.5 THz. An analysis from the band structure suggests that it is plausible to match P1 to the optical mode at the Z point while P2 can be matched with phonon modes at the Γ point which are close in energy (almost degenerate). P3 is an optical mode at the Γ point. This is clearly in good agreement with the experimental result of Figure A.5, where absorption coefficient converges to 0.5 THz and 1.1 THz (almost degenerate peaks), when extrapolated to 0 K. The phonon eigenvalues at the Γ point from Density-functional Perturbation Theory (DFPT) calculations for a $2 \times 2 \times 1$ supercell are found to be 1.11 THz, 1.13 THz and 1.26 THz for the three low energy phonon modes. The symmetry of the three modes at the Γ point is found to be A'' , A' , and A' , all of which are IR active. In order to observe the effect of the volume change, we

computed the phonon DOS with a volume change of $+1.5\% V_0$ and $-1.5\% V_0$ shown as red and green solid lines (Figure A.5), respectively. The octahedral tilting plays a significant role in the phase transition of the BIT system. We have also explored the effect of the octahedral tilting on the phonon DOS by changing the monoclinic angle α_0 to $+0.04\% \alpha_0$ and $+0.07\% \alpha_0$, as shown in Figure A.5 in blue and magenta solid lines, respectively. These theoretical calculations suggest that the phonon peaks are shifted to lower frequencies for the models deviating from the ground state monoclinic structure. The peak P2 appears to split the constituting phonon modes by about 0.2 THz when the volume is increased due to atomic rearrangement in a relaxed lattice. On reduction of the volume to $0.985 V_0$, the phonons were found to exhibit hardening behavior. The analysis of energy as a function of mode amplitude for the three low energy phonon modes, for the models with increased lattice angle with respect to the ground state monoclinic value (α_0), suggested two soft phonons. These results indicate soft phonons in BiT which appear with changes in both volume and the octahedral tilting. The atomic rearrangement accommodating these changes could lead to the anharmonicity in the interatomic bonds, as observed in the PDF analysis of Figure A.5.

The phonons at P2 and P3 of Figure A.55, show vibration of ions in the perovskite and fluorite blocks. The correlated motion of atoms within each block, which are out-of-phase among each other, is shown in the gray and green regions in Figure A.5c. The out-of-phase oscillations of the lattice blocks could potentially lead to the deviation from the monoclinic towards tetragonal phase (Figure A.5d). The structural change could be described using the lattice parameter transformation $a < b \rightarrow a' = b'$, such that the lattice parameter of the tetragonal phase is $a' (=b') = a/\sqrt{2}$ (Figure A.5e). This mechanism is consistent with the theoretical findings of Ref. [223], where it is suggested that two unstable E_u modes in BiT, one involving the motion of fluorite layers in a direction relative to the perovskite $(\text{TiO}_6)^{8-}$ blocks and the second mode involving the motion of the Bi ions in the perovskite A site with respect to the perovskite blocks, are responsible for the phase change. The atomic displacements in the fluorite layers are larger than in the perovskite layers for the three calculated modes in our study. Thus, we underline that the chemical nature of large cation in the fluorite layers in the Aurivillius family and similar layered oxides is crucial for structural transformations. We note, however, that the theoretical tools used here could have certain limitations while applying for more complicated structures. Firstly, the quasi-harmonic approximation is not appropriate for larger scale volume and/or angular variations. In addition, this approach may not be applicable to accurately determine phase transformation temperatures in strongly correlated systems. Future improvements on the current theoretical foundations will therefore be necessary to tackle more complex systems.

A.4. Conclusions

In summary, we have probed the dynamics of soft phonon modes and its role in the structural transformations on highly textured (00l) oriented $\text{Bi}_4\text{Ti}_3\text{O}_{12}$ using THz frequency-domain spectroscopy. The results from the THz frequency-domain spectroscopy have revealed three low frequency soft phonon modes, which have been supported from first-principles study. The anharmonicity of the Bi–O bonds plays a leading role in these low frequency phonon modes with majority of contribution to the phonon density of states comes from the Bi atoms. The fundamental understanding about various factors affecting phonon dynamics and structural changes described here provides useful information in designing tailored phase transition and functionality (e.g. ferroelectric and thermal properties) of layered-structure ferroelectric materials.

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