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# Investigating Hydration Dynamics and Protein Collective Motions by High-Precision Dielectric Spectroscopy

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## ABSTRACT

Biological processes often take place at surfaces of proteins, where the dynamic and structural properties of aqueous solvents are modified. Information about solvent properties including hydration dynamics and structure, and protein collective motions can be obtained by measuring directly the dielectric response in the megahertz to terahertz frequencies of aqueous protein solutions. Due to the strong absorption of water in this frequency range, the experiment is challenging. Our home built dielectric spectrometer using a vector network analyzer together with frequency extenders allows us to perform the experiment in a wide range of frequency from megahertz to terahertz with a high dynamical range up to 120 dB. A detailed investigation of the dielectric response has revealed the hydration structure including the tightly, loosely bound layers and the number of water molecules in each hydration layer. These water molecules relax with different time constants at different temperatures. As a result, the dynamics of hydrated protein is also probed at different temperatures. Understanding the hydration structure and dynamics of lysozyme in biological conditions can explain the enzymatic activities of biomolecules.

**Keywords:** Dielectric relaxation spectroscopy, Megahertz, Terahertz, Protein dynamics, Hydration dynamics

## INTRODUCTION

Protein molecules exist in the aqueous environment, from that all functions of proteins are performed through the interaction of the molecules with the surrounding solvent molecules. Functions of proteins are enforced by the changing of the structure and shapes,[1-3] which are based on the levels of molecular movements including molecular reorientation, collective motion or conformational change, and the atomic/molecular oscillating around their neutral positions.[4-7] Naturally, proteins and other bio-molecules function in the aqueous environment.[8, 9] With the small size and a large electrical dipole moment, water produces a strong interaction with surface charges of proteins,[10] forming hydration layers. Thus, water molecules directly involve and dominate protein dynamics. Therefore, the structure and dynamic of the hydration layers have to be probed together with the protein dynamics.

The megahertz to terahertz dielectric spectroscopy can probe directly the hydration water as well as the dynamics of biomolecules in solution at biological conditions.[4, 11-15] However, the number of terahertz ready devices are limited. Typically, the terahertz radiation source can either get by upwarding frequency from electronic sources or downwarding frequency from light sources.[16] Moreover, water is a strongly absorbing in the gigahertz to terahertz frequencies,[13, 14, 17] thus, absorption measurements of aqueous solutions require both a high sensitivity terahertz detector and a strong terahertz radiation source. We have successfully developed a dielectric spectrometer with the frequency spanning from megahertz to terahertz with high effective dynamical ranges up to 120 dB.[12, 18, 19] In this paper we present dielectric response results of lysozyme aqueous solutions. Lysozyme is a polypeptide chain protein with the molecular weight of 14.3 kDA. Lysozyme is an enzyme found in bodily secretions including human milk, saliva, mike, and in many biological organisms from bacteria and fungi. Its functions as an antimicrobial enzyme by cleaving glycosidic bonds in peptidoglycans in bacterial cell walls.[20] In the experiment, lysozyme was dissolved in water with the concentration of 10 mM.[6, 7]

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## EXPERIMENTAL SETUP

To probe the hydration dynamics and structure together with collective motions of bio-molecules in an aqueous environment, we have built a frequency-domain dielectric spectrometer covering the spectra range from 10 MHz to 1.12 THz. In order to overcome the strong absorption of water in the measured frequencies, our system has been developed to achieve a high dynamic range up to 120 dB compared to a commercial terahertz time-domain spectrometer with a dynamic range of 60 – 70 dB.[12]

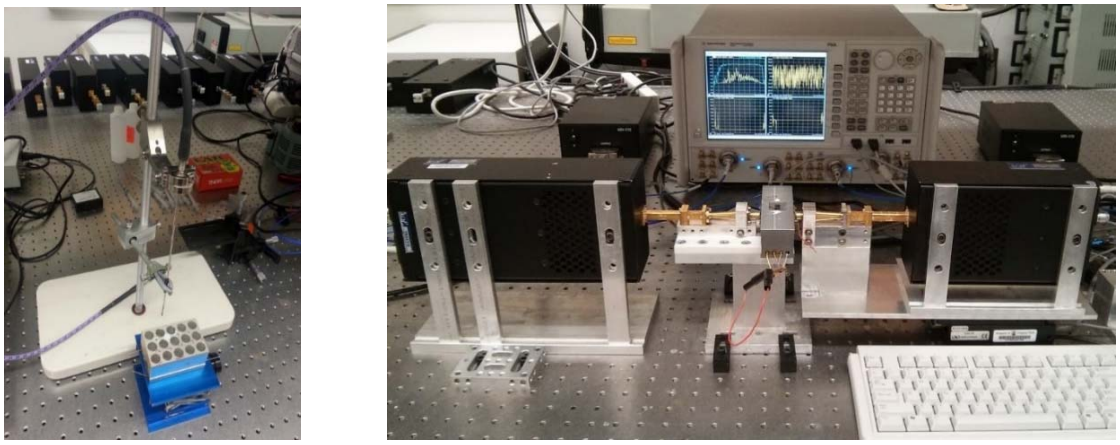

**Figure 1:** The megahertz to terahertz dielectric spectroscopy setup includes (left) coaxial probe experiments, and (right) a terahertz frequency extender system.

The megahertz to terahertz dielectric spectrometer consists of a commercial Vector Network Analyzer (VNA) from Agilent (N5225 PNA) and frequency extenders developed by Virginia Diodes Inc. (VDI). The Agilent VNA provides electromagnetic wave in the frequency range between 10 MHz to 50 GHz with a power up to 20 mW. The radiation from VNA is used to directly measure the dielectric response of aqueous solutions.[21] Higher frequencies up to the terahertz region can be obtained with VDI frequency extenders.[12] To cover the terahertz region from 60 GHz to 1.12 THz, seven different pairs of frequency multipliers have been employed including WR10, WR6.5, WR5.1, WR3.4, WR2.2, WR1.5 and WR1.0 bands. A variable path-length sample cell setup[12] has been employed to measure the absorption and refractive index of aqueous solutions at the terahertz frequencies (Fig. 1). The absorption process of the electromagnetic wave passing through a sample is described by the Beer's law:

$$I(l, \nu) = I_0(\nu).\, e^{-\alpha(\nu).l} \tag{1}$$

where $I_0$, $I$, $l$, $\nu$, $\alpha(\nu)$ are the intensity before and after the sample, the thickness of the sample, measured frequency, and absorption coefficient of the solution, respectively. The thickness of the sample or the distance between two transparent windows of the setup can be precisely controlled with an error of less than 50 nm by using an ultra-precision linear translation stage (XMS160) from Newport. In parallel, the phase shift $\theta(l, \nu)$ as a function of the thickness of the samples has been obtained.

To control the temperature with high accuracy, high power resistors and liquid cooling sets integrated into the sample cell are controlled and monitored by the 336 Lake Shore Temperature Controller with a platinum sensor (PT-111). The setup allows us to work on a broad range of temperature from -20 ºC to 90 ºC with an error of ± 0.02 ºC.

The intensity and phase shift measured from our setup can be used to calculate the complex index of refraction, $n^*(\nu)$, of a solution:

$$n^*(\nu) = n(\nu) + i\kappa(\nu) \tag{2}$$

where $n(\nu)$ is refractive index and $\kappa(\nu)$ is the extinction coefficient. The extinction coefficient can be calculated from the absorption coefficient, $\alpha(\nu)$:

$$\kappa(\nu) = \frac{\alpha(\nu)\cdot c}{4\pi\nu} \tag{3}$$

with $c$ is the speed of light.

It is more convenient to present the complex index of refraction in the form of the dielectric constant, $\varepsilon^*(\nu)$, of the solution:

$$n^*(\nu) = \sqrt{\varepsilon^*(\nu)} = \sqrt{\varepsilon'(\nu) + i\varepsilon''(\nu)} \tag{4}$$

where the real part $\varepsilon'(\nu)$ stands for the dielectric dispersion, and the imaginary part $\varepsilon''(\nu)$ stands for the dielectric loss component of the solution. The dielectric dispersion presents the energy stored per unit volume, which is related to the refractive index. The dielectric loss represents the energy dissipated per unit volume of the solution and is related directly to the absorption coefficient. They can be equivalently calculated from each other by following equations:

$$n(\nu) = \sqrt{\frac{\sqrt{\varepsilon'(\nu)^2+\varepsilon''(\nu)^2}+\varepsilon'(\nu)}{2}} \tag{5}$$

$$\kappa(\nu) = \sqrt{\frac{\sqrt{\varepsilon'(\nu)^2+\varepsilon''(\nu)^2}-\varepsilon'(\nu)}{2}} \tag{6}$$

Thus, we have:

$$\varepsilon'(\nu) = n^2(\nu) - \kappa^2(\nu) \tag{7}$$

$$\varepsilon''(\nu) = 2n(\nu).\kappa(\nu) \tag{8}$$

## RESULTS AND DISCUSSION

The high sensitivity of our dielectric spectroscopy allows us to study the dynamics of biomolecules in aqueous solutions. Figure 2 shows the absorption coefficient and refractive index of pure water and 10 mM lysozyme solution at 25 °C as a function of frequency. The absorption coefficient increases monotonically with rising frequency, whereas the refractive index gently decreases. Comparing water and 10 mM lysozyme solution, both absorption coefficient and refractive index of the lysozyme solution are lower than those of pure water. This indicates that the present of lysozyme in water changes optical properties of the solution. The biomolecules take place of water molecules, reducing the number of water molecules in the solution. The absorption of biomolecules is lower than that of water, thus, the absorption of the solutions is reduced. Also, water form hydration layers around biomolecules due to the interaction of bimolecular surfaces with water molecules. These water molecules have a strong bond with the surface and relax with a slower time constant compared with those of bulk water.

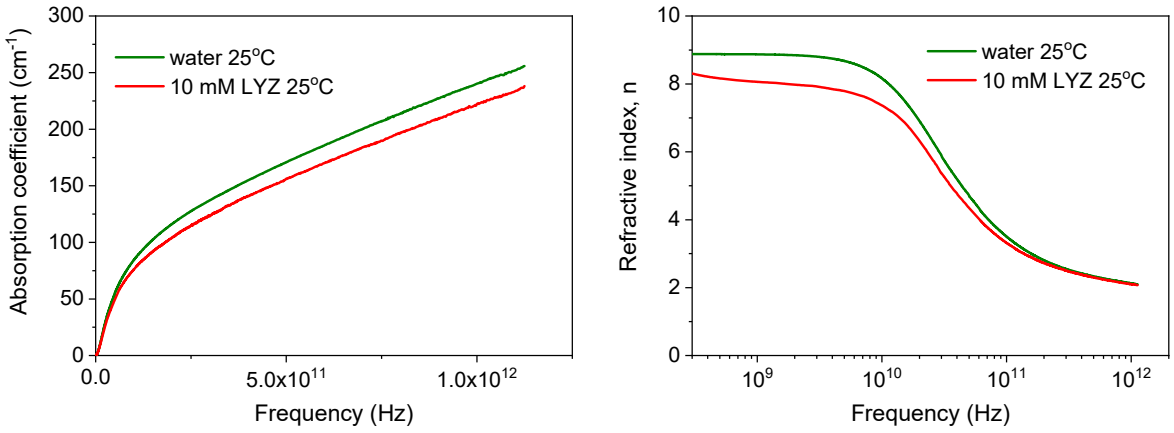


**Figure 2:** (left) Absorption coefficient and (right) refractive index of water, and 10 mM lysozyme solution at 25 °C.

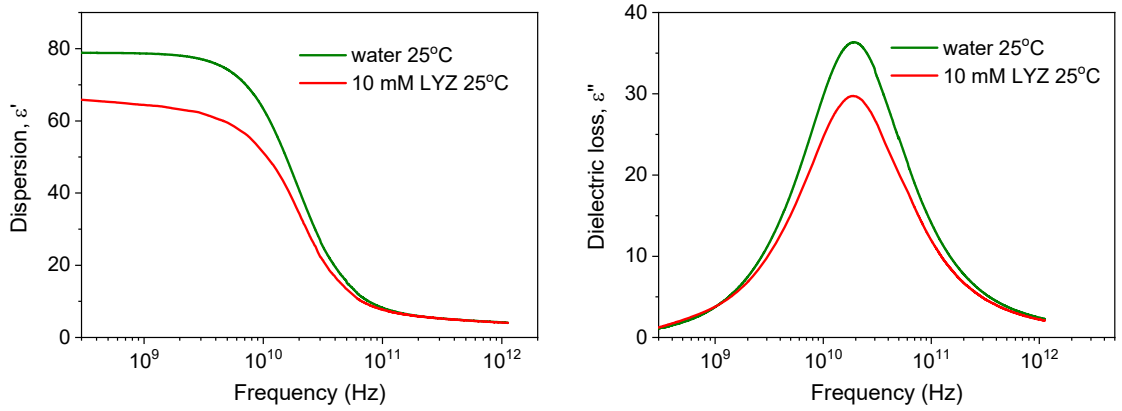


**Figure 3:** Dielectric response of water and 10 mM lysozyme solution at 25°C, including the dielectric dispersion, $\varepsilon'(\nu)$, and dielectric loss, $\varepsilon''(\nu)$.

To explore the interaction of the megahertz to terahertz electromagnetic wave with the biological solution, we presented dielectric response in Fig. 3. The dielectric dispersion, $\varepsilon'(\nu)$, for both water and the lysozyme solution reduces with increasing frequency, and values for the lysozyme solution are lower. The dielectric loss, $\varepsilon''(\nu)$, shows a complex behavior. The frequency of the main peak centered at ~20 GHz appears in both water and the lysozyme solution, but the maximum for the lysozyme solution is lower when compared with that of water. Taking a closer look at the gigahertz frequencies, values of dielectric loss of the lysozyme solution is higher than those of water. To explore the dielectric response at the megahertz to gigahertz frequencies, Debye-type relaxations is sufficient to consider. At the molecular level of the interaction between protein and water molecules, water molecules in protein solutions can be divided into several specific types named tightly bound, loosely bound, and bulk water.

$$\varepsilon^*(\nu) = \varepsilon_\infty + \varepsilon_m + \frac{\Delta\varepsilon_{\mathrm{TB}}}{1+i2\pi\nu\tau_{\mathrm{TB}}} + \frac{\Delta\varepsilon_{\mathrm{LB}}}{1+i2\pi\nu\tau_{\mathrm{LB}}} + \frac{\Delta\varepsilon_{\mathrm{B}}}{1+i2\pi\nu\tau_{\mathrm{B}}} \tag{9}$$

where $\varepsilon_\infty$ is the contribution to the total dielectric response from modes at high frequencies, $\varepsilon_m$ is the dielectric response for the reorientation process of the macromolecules in the solution, typically in the megahertz frequencies; $\Delta\varepsilon_{\mathrm{TB}}$, $\Delta\varepsilon_{\mathrm{LB}}$, $\Delta\varepsilon_{\mathrm{B}}$, $\tau_{\mathrm{TB}}$, $\tau_{\mathrm{LB}}$, $\tau_{\mathrm{B}}$, are the dielectric strength and relaxation times of water molecules in the tightly bound, loosely bound layers, and bulk water, respectively. From the fitting parameters of the contribution of water molecules in different layers based on the Debye's model (Eq. 10), we can estimate the total number of water molecules affected by the present of lysozyme in solution. As a result, from the Debye fitting, we estimate around 700 ± 50 water molecules forming the tightly and loosely bound layers.

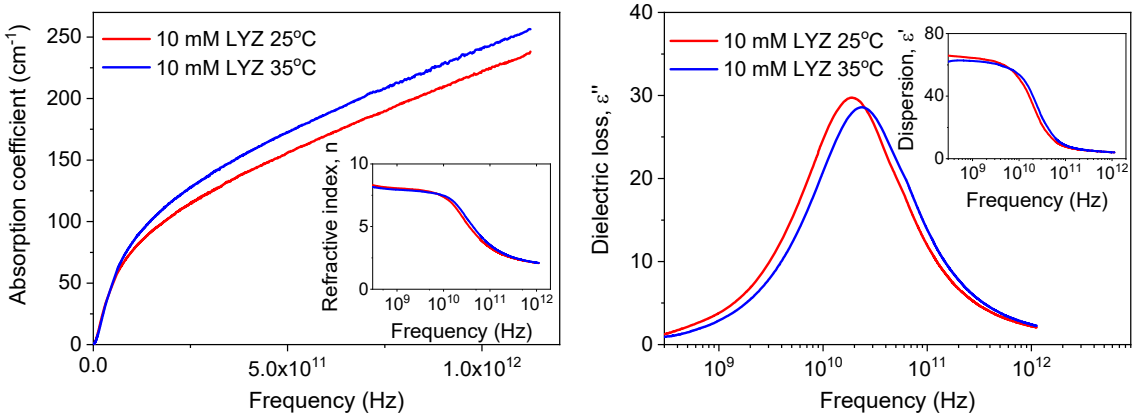


**Figure 4:** (left) Absorption coefficient and refractive index (in the inset), and (right) dielectric loss and dielectric dispersion (in the inset) of 10 mM lysozyme solution at 25 and 35 °C.

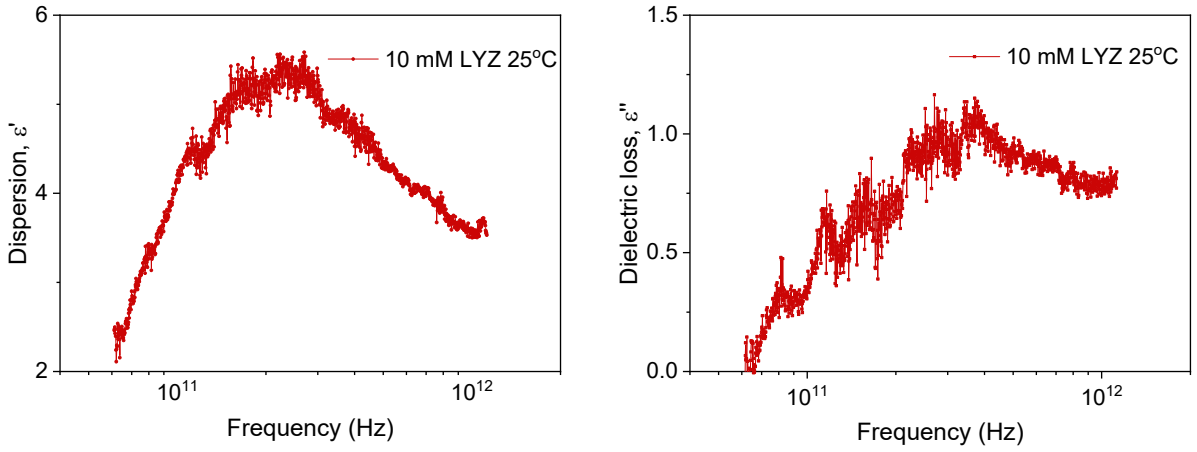


**Figure 5:** Dielectric spectra of hydrated lysozyme at 25 °C in the terahertz frequencies resulted from the Bruggeman effective medium theory approximation.

To study the temperature effect on the water and lysozyme dynamics in solution, we performed the experiment at 25 and 35 °C. The results are shown in the Fig. 4. Absorption coefficient is higher at higher temperature on the whole frequency range, and the refractive index at higher temperature is lower at lower frequencies then higher at higher frequencies. The dielectric response plots show a clear temperature effect. The dielectric loss is lower at higher temperature. The peak of the dielectric loss shifted to higher frequencies at higher temperature means that the reorientation of water molecules in the solution is faster at higher temperature. With a higher dynamic, water molecules can escape easily from the surface of lysozyme, thus, lowering of the total number of water molecules trapped in hydration layers. The Debye' fitting confirms that a total of 670 ± 50 water molecules are captured in the tightly and loosely bound layers of a lysozyme molecule at 35 °C.

At the terahertz frequencies, the electromagnetic wave can probe collective motions of biomolecules in aqueous solutions. Water molecules interact directly with lysozyme surface form tightly bound water layer. These water molecules constitute the hydrated protein, and cannot easily move in and out. To extract the dielectric response of hydrated lysozyme, the effective medium theory has been employed. Lysozyme molecules were assumed in a spherical shape with a radius of $R_{\mathrm{LYZ}}$, and water molecules form a tightly hydration shell with a thickness of $d$. Then, the volume fraction, $f$, of hydrated lysozyme in solution can be calculated:

$$f = \frac{N_{\mathrm{LYZ}}}{V}.\frac{4\pi}{3}(R_{\mathrm{LYZ}} + d)^3 \tag{10}$$

where $N_{\mathrm{LYZ}}$ and $V$ are number of lysozyme molecules and the total volume of the solution, respectively. The size of hydrated lysozyme is much smaller than the probing wavelength in the experiment. For a wide range of lysozyme concentration, the Bruggeman model is the best fit for this approximating task.[22, 23]

$$\varepsilon^*_{\mathrm{LYZ}}(\nu) = \frac{2\varepsilon^{*\,2}_{\mathrm{SOL}} + (3f-2)\varepsilon^*_{\mathrm{SOL}}\varepsilon^*_{\mathrm{WAT}}}{(3f-1)\varepsilon^*_{\mathrm{SOL}} + \varepsilon^*_{\mathrm{SOL}}} \tag{11}$$

where $\varepsilon^*_{\mathrm{LYZ}}$ is the complex dielectric response of hydrated lysozyme, $\varepsilon^*_{\mathrm{SOL}}$ and $\varepsilon^*_{\mathrm{WAT}}$ are the measured dielectric response of the 10 mM lysozyme solution and pure water, respectively.

Results of the dielectric response of hydrated lysozyme using the Bruggeman effective medium theory approximation obtained by varying the volume fraction are shown in Fig. 5 at 25 °C. Calculating from the value of the volume fraction, $f$, we have estimated 195 ± 30 water molecules in the first hydration shell or tightly bound layer, which is less than one layer of water molecules around a lysozyme molecule.[6, 7]

The number of water molecules calculated from the effective medium theory approximation at the terahertz frequencies is expected to be lower than that from the Debye-type relaxation in the gigahertz frequency range. These water molecules are located in the tightly bound layer, and integrated strongly to lysozyme.

We have carried out the high-precision dielectric spectroscopy from megahertz to terahertz frequencies of lysozyme in aqueous solution. A detailed investigation of the dielectric response has uncovered the hydration structure and dynamics, including the tightly, loosely bound layers, and the number of water molecules in each hydration layer. These water molecules relax with different time constants at different temperatures. As a result, the dynamics of hydrated protein is also probed at different temperatures. Understanding the hydration structure and dynamics of lysozyme in living conditions can explain the enzymatic function of biomolecules at different temperatures.

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