Studying Protein Dynamics in Aqueous Solutions through Linear and Non-linear THz Spectroscopy

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Abstract— **Direct absorption spectroscopy is used to determine the absorption coefficient of protein solutions across the spectrum from 0.1 to 3 THz. The spectra are analyzed with respect to their dependence on solution properties, protein size and protein geometry. Besides these direct absorption measurements, a non-linear two-color experiment is developed which is sensitive solely to the chirality based contribution from proteins.**

I. Introduction And Background

Biopolymers such as proteins exhibit structural dynamics over a very broad range of frequencies. Large scale motions connected to their functional properties have been shown to exist in the THz frequency range.[1,2] The application of direct absorption spectroscopy[3] for the study of these motions in the natural environment of the biopolymers, aqueous solutions, comes with the added difficulty that water itself absorbs rather strongly at these frequencies. In order to determine the protein contribution to the absorption of the solution, we need to measure the absorption coefficients of the protein solution and its reference solution with high precision. This is achieved by combining a strong THz source, the UCSB Free-Electron Laser, and a highly sensitive THz detector, a helium cooled bolometer, with a precision solution-thickness scanning setup**.** The resulting data can be analyzed with Beer's law in order to yield the absorption coefficient of the solution with high precision.

Beyond this direct method, a non-linear spectroscopy approach to study global protein dynamics is developed. Due to the chiral symmetry that biopolymers exhibit, their solutions produce a non-vanishing contribution to the second-order non-linear polarization governing sum/difference frequency generation. When a protein solution is simultaneously excited at an electronic transition at optical wavelengths and by THz radiation, sidebands to the optical frequency are generated. The strength of these sidebands reflects the vibrational mode structure of the protein close to the chromophore which often participates directly in the function of the protein.

II. Results And Discussion

The measurements discussed here have been taken on solutions containing the protein lysozyme. Direct absorption measurements across the THz spectrum of high concentration solutions (25 % w/w) of the native protein[4] with a radius of gyration of 1.53 nm show the following features. The overall absorption starts to rise up at about 0.4 THz and then levels off into a plateau at about 2 THz. These features compare reasonably well with theoretical mode calculations of the protein[4].

Well below half a THz the situation is more complicated. In the absorption measurements, one measures the absorption strength of both the protein in buffer solution and the pure buffer solution. In order to do this right, care has to be taken to measure exactly, by weight, how much buffer was put into the protein solution. Then the absorption coefficient of the pure buffer is scaled by this percentage and subtracted from the protein solution absorption. Surprisingly, this leads to a negative value for the protein absorption in the low frequency region (<0.25 THz). An explanation for this observation comes in the form of water of hydration. The water in the direct vicinity of the protein will exhibit different dynamics than free water molecules[5,6,7,8]. This can shift oscillator strength from lower (less stiff) to higher frequencies (stiffer). Combining this explanation with the experimental observation that the negative absorption at the lower frequency end appears frequency independent leads one to conclude that the low frequency behavior is dominated by the water of hydration and the protein contribution is negligible. Under the assumption of zero absorption for both the protein and the water of hydration one recovers a number of water molecules per protein of the hydration shell that is at the lower end of values determined from other approaches.

The rise in absorption strength with increasing frequency mimics the behaviour of a disordered solid with randomly distributed charges. In the latter system, the absorption strength basically follows the density of states (DOS) of the vibrational modes. To test whether protein solutions show a similar characteristic[9], in addition to the native protein (3D like DOS), solutions of lysozyme in its denatured state (1D like DOS) are investigated. Reduced denatured lysozyme is made[10] from the native source and then dialyzed against the same pH2 buffer as the native solutions. In its modified state, lysozyme has a radius of gyration of about 3.8 nm, about 2.5 times that of the native state. The concentrations of both types of solutions, native and modified, are determined by UV absorption spectroscopy. The change in protein conformation is confirmed both by CD and 1D NMR measurements. Working with proteins in their denatured state brings with it the difficulty that their solubility limit is much lower than that of the native state even far from the isoelectric point (pH11.4). For the modified lysozyme solutions a maximum concentration close to 4 % w/w could be achieved. This brings with it a significant loss of signal strength from the protein. Figure 1 shows the absorption values for the buffer solution, the native lysozyme solution (3.9 % w/w) and the buffer absorption scaled by its content in the lysozyme solution at THz frequencies. The absorption in all solutions is clearly dominated by the water contribution and emphasizes that the

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absorption needs to be measured with very high precision.

The main question to be addressed here, is whether the lysozyme geometry significantly influences the THz

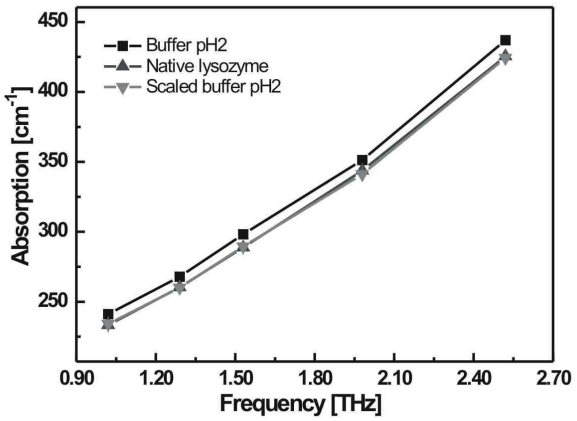

Figure1: THz absorption coefficients for a native lysozyme solution (3.9 % w/w) the pure buffer and the buffer scaled to the volume concentration in the lysozyme solution

absorption of the protein. To that effect, Figure 2 shows the absorption contribution determined for three different solutions at about equal concentrations. The native lysozyme (3.9 % w/w) solution from above, a similar native lysozyme (3.9 % w/w) solution with part of the buffer made up from positively charged TRIS buffer and a modified lysozyme solution (3.7 % w/w).

The data shows no significant difference between the three solutions at most frequencies. The difference at 2.5 THz for the native solution might indicate a systematic problem at this frequency. Due to the rapid increase in water absorption, both the absolute thickness and the thickness changes over which the absorption is determined according to Beer´s law become rather small at high THz frequencies and thus more prone to errors. However, the rest of data shows no dependence on the geometry of the protein.

Similar measurements performed at lower THz frequencies are complicated by the additional contribution from the water of hydration which results in a negative absorption coefficient. In order to determine this molecules of water per molecule of protein number, NMR measurements on frozen solutions similar to those in Ref. 8 (Sec. II C) are under way.

Due to the difficulties involved in direct THz absorption measurements on protein solutions as outlined in the discussion above, it is desirable to develop a measurement approach that results in a signal solely from the biomolecules. The chiral symmetry of the biomaterial quite naturally leads the way to the technique of chiral sum and difference frequency generation[11]. In our approach, the THz beam is focused onto a solution surface which sits right above a metal grating. The frequency mixing occurs in the evanescent field of the wavefront modulated THz beam in the solution through which a visible laser beam is guided just above the grating.

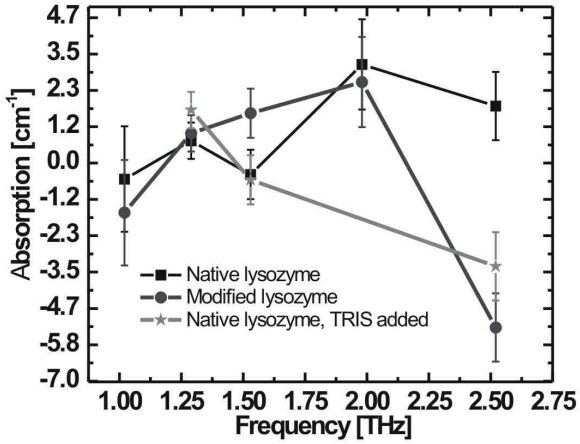

Figure2: THz absorption coefficients for native lysozyme (3.9 % w/w), modified lysozyme (3.7 % w/w) and native lysozyme (3.9 % w/w) with added TRIS buffer.

Through variation of the grating period phase matching conditions across the whole THz band can be achieved. The setup is currently analyzed with non-linear crystals especially with respect to the achievable signal strengths compared to the expectations from chiral sum and difference frequency generation performed in the IR[11].

References

[1] F. Rosca *et al*., "Investigations of Anharmonic Low-Frequency Oscillations in Heme Proteins" in *J. Phys. Chem. A*, *106,* 3540-3552, 2002.

[2] J. R. Knab, et al., "Terahertz Measurements of Protein Relaxational Dynamics", *Proceedings of the IEEE*, 95, 8, 1605 – 1610, 2007

[3] D. F. Plusquellic *et al*, "Applications of terahertz spectroscopy in biosystems", *Chem. Phys. Chem*., 8, 2412-2431, 2007

[4] J. Xu, K. W. Plaxco, and S. J. Allen, "Collective dynamics of lysozyme in water: Terahertz absorption spectroscopy and comparison with theory", *J. Phys. Chem. B*, vol. 110, pp. 24 255–24 259, 2006.

[5] S. Ebbinghaus *et al,* "An extended dynamical hydration shell around proteins", *PNAS*, 104, 20749-20752, 2007

[6] CF. Zhang, S. M. Durbin, "Hydration-induced far-infrared absorption increase in myoglobin", *J. Chem. Phys. B*, 110,23607-23613, 2006.

[7] A. Dominguez-Vidal et al.," Probing intermolecular interactions in water/ionic liquid mixtures by far-infrared spectroscopy", *J. Phys. Chem. B*, 111, 4446-4452, 2007

[8] I. D. Kuntz, W. Kauzmann,"Hydration of Proteins and Polypeptides", *Adv Protein Chem.* 28:239-345, 1974.

[9] E. Castro-Camus, M.B. Johnston,"Conformational changes of photoactive yellow protein monitored by terahertz spectroscopy", *Chem. Phys. Lett*., 455, 289-292, 2008

[10] K. Shiraki, eta al, "Trifluoroethanol-induced stabilization of the alpha-helical structure of beta-lactoglobulin: implication for non-hierarchical protein folding", *J. Mol. Biol.* 245, 180–194, 1995.

[11] M.A. Belkin et al., "Resonant sum-frequency generation in chiral liquids", *Optical Materials* 21, 1–5, 2002

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