Anisotropic Absorption Measurements Reveal Protein Dynamical Transition in Intramolecular Vibrations

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***Abstract*—Modeling has predicted that intramolecular structural vibrations enables proteins to access functionally important structural change. We show that the vibrational density of states and the isotropic absorption in the terahertz range are only weakly dependent on the protein functional state for several bench marking proteins. At the same time the direction of motions changes dramatically with functional state and with a resulting impact on the anisotropic absorption. Our anisotropic THz microscopy (ATM) measurements confirm this sensitivity. Here we apply the technique to the question of whether the protein dynamical transition (DT) is important to protein function. We find a strong anisotropic resonance at 70 cm^{-1} rapidly increases in strength at temperatures above the DT. As these intramolecular vibrations enable protein structure to change conformation, the results suggest function will cease below DT for those proteins that require large scale conformational change.**

I. Introduction

Terahertz spectroscopy has been applied to the measuring of intramolecular dynamics in biomolecules. Standard terahertz spectroscopy has found a broad absorbance for typical protein samples such as lyophilized powders and solutions. Recently we have found Anisotropic Terahertz Microscopy (ATM) of protein crystals measures narrow anisotropic resonances that are sensitive to the protein functional state [1,2]. Protein crystals provide the necessary alignment of the large macromolecules for polarization dependent measurements. These molecular crystals consist of an ordered array of protein molecules (~>100 atoms per molecule) and approximately 50% water by volume. This is equivalent to a hydration value of 0.73 grams water to grams protein. Extensive studies on a variety of proteins have found that only 0.30 hydration is necessary to attain fully solvated structure and dynamics [3,4].

II. RESULTS

A long standing controversial phenomenon in protein dynamics has been the protein dynamical transition, which is the name given to the rapid increase in the mean squared atomic displacement at ~200-220K for minimally hydrated proteins. For some proteins it appears the transition corresponds to the turn on of function, while for others there is no significant change in function at the transition. By using ATM we find that the dynamical transition occurs in the long range vibrations, suggesting that the transition is important to the function of those proteins that require large structural changes that are enabled by these vibrations. In Fig. 1 we show representative ATM measurements. The precise technique used for these measurements rotates the THz polarization rather than the sample. This technique is referred to as Polarization Varying ATM or PV-ATM. Below the dynamical transition, there is a relatively weak resonance near 70 cm^{-1}, which rapidly grows in strength as the temperature increases above 220K. We suggest the long range motions require a critical population of thermally activated water motions surrounding the protein to enable the collective vibration of the protein backbone, leading to the temperature dependence in the anisotropic absorption.

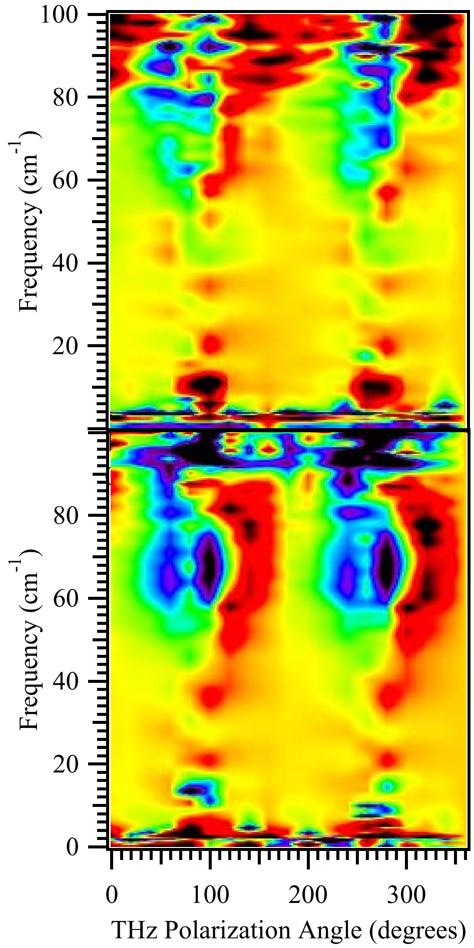

Fig. 1. PV-ATM measurements of CEWL tetragonal crystal as function of temperature. Top data for 190 K and bottom data for 245K.

References

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