Protein Thermal Conductivity Measured in the Solid State Reveals Anharmonic Interactions of Vibrations in a Fractal Structure

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ABSTRACT: Energy processes and vibrations in biological macromolecules such as proteins ultimately dictate biological, chemical, and physical functions in living materials. These energetic vibrations in the ribbon-like motifs of proteins interact on self-similar structures and fractal-like objects over a range of length scales of the protein (a few angstroms to the size of the protein itself, a few nanometers). In fact, the fractal geometries of protein molecules create a complex network of vibrations; therefore, proteins represent an ideal material system to study the underlying mechanisms driving vibrational thermal transport in a dense, fractal network. However, experimental studies of thermal energy transport in proteins have been limited to dispersive protein suspensions, which limits the knowledge that can be extracted about how vibrational energy is transferred in a pure protein solid. We overcome this by synthesizing solid, water-insoluble protein films for thermal conductivity measurements via time-domain thermoreflectance. We measure the thermal conductivity of bovine serum albumin and myoglobin solid films over a range of temperatures from 77 to 296 K. These temperature trends indicate that anharmonic coupling of vibrations in the protein is contributing to thermal conductivity. This first-ever observation of anharmonic-like trends in the thermal conductivity of a fully dense protein forms the basis of validation of seminal theories of vibrational energy-transfer processes in fractal objects.

SECTION: Energy Conversion and Storage; Energy and Charge Transport
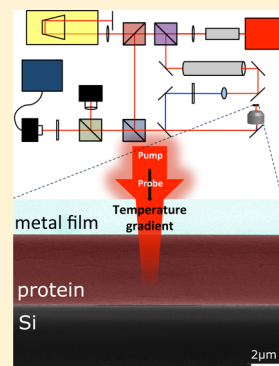
Energy processes and vibrations in biological macro-molecules such as proteins ultimately dictate biological, chemical, and physical functions in living materials. The vast functional duties of proteins − from structural building blocks to molecular recognition, catalysis, and energy transduction[1−5] − are ultimately governed by the energetic vibrations of the molecules. Thus, the connection between protein structure and the pathways of energy flow is a fundamental concern that dictates protein functionality and reactivity. These factors drive a protein's applicability and functionality for both native and artificial applications.[6]

In addition to their fundamental importance in bioprocesses, proteins present a virtually unexplored avenue to study the fundamentals of thermal transport in naturally occurring nanostructured, percolating networks. To date, thermal conduction in biological materials has often been explored in the context of amorphous solids and evaluated through comparison with some variation of the so-called "minimum limit to thermal conductivity"[7−12] at elevated temperatures above ~50−100 K as well as data from atomistic molecular dynamics simulations. However, the ribbon-like motifs of

proteins lead to self-similar structures and fractal-like objects over a range of length scales of the protein (a few angstroms to the size of the protein itself, a few nanometers)[6,13,14] that can be fundamentally different from those found in polymers or amorphous solids.[15,16] In these protein systems, vibrations of the fractal structure can add additional complexities to understanding vibrational transport. Therefore, proteins represent an ideal material system to study the underlying mechanisms driving vibrational thermal transport in a fractal solid.

The nature of the thermal conductivity of proteins was studied computationally by Yu and Leitner via molecular dynamics simulations.[13,17,18] They determined that anharmonic vibrational interactions among protein molecules can open up an additional channel for thermal conduction in a protein, in line with previous theories of thermal conduction in fractal objects.[19−22] In two separate works, they also evaluated the

thermal conduction in the case of both spectral[13] and gray[18] mean free paths of protein vibrations. Unfortunately, no conclusive experimental evidence exists to validate the results from Yu and Leitner nor the aforementioned theoretical work, leaving unanswered questions regarding the role of anharmonicity in the thermal conductivity of a protein and a fractal object.

In response, we synthesize a series of solid, water-insoluble protein films on substrates to provide a platform to measure the thermal conductivity of these fractal structures. We measure the thermal conductivity of bovine serum albumin (BSA) and myoglobin. Unlike previous works on the thermal conductivity of proteins,[23−26] we study solid, insoluble protein structures as opposed to proteins suspended in water or water-saturated proteins. This allows us to directly measure the thermal transport in the network of protein molecules over a range of temperatures without complications from the solution.[27,28] Using time-domain thermoreflectance (TDTR), we measure the thermal conductivity of these proteins from 77 to 296 K. The temperature trends are consistent with previous computational works that show that anharmonic coupling of localized vibrations in a fractal protein structure contributes to thermal conductivity.

Water-insoluble protein films were prepared by adapting a previously described method.[29] First, proteins were dissolved in phosphate-buffered saline (PBS; pH 7.4) to a final concentration of 3% (wt/vol). This solution was reacted with 0.2 M ethylene glycol diglycidyl ether (EDGE) for 24 h at room temperature. EDGE is a polyepoxy cross-linking reagent that produces protein intermolecular cross-links between amine-containing residues such as histidine and lysine while maintaining overall protein conformation. The reaction was dialyzed for 24 h against DI water using a 7 kDa molecular weight cutoff membrane. This solution was either drop-cast or spin-coated onto oxygen plasma-treated substrates (borosilicate coverslips and silicon) to achieve thicknesses spanning micrometers to nanometers. These procedures resulted in fully dense protein films, as shown in Figure 1. For data collection with TDTR, we coated all protein samples with a thin Al film, as discussed in more detail later.

The physical structures of constituent proteins in these films were characterized using circular dichroism (CD) spectroscopy. Figure 2 shows the far-UV CD spectra of a BSA film spin-coated on a quartz substrate where characteristic double minima at 208 and 222 nm are indicative of intact-helix that
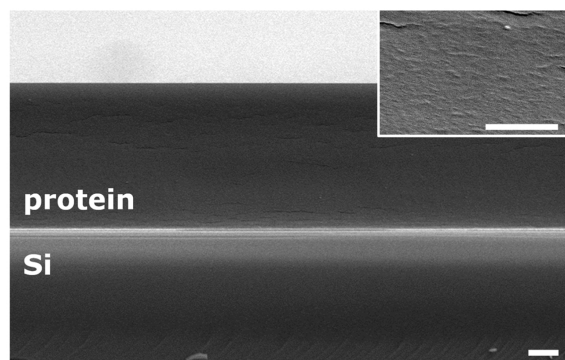

Figure 1. Scanning electron microscopy cross-section image of a BSA protein film drop-cast onto a silicon substrate. Inset shows higher magnification of the solid protein film. Scale bars = 1 μm.
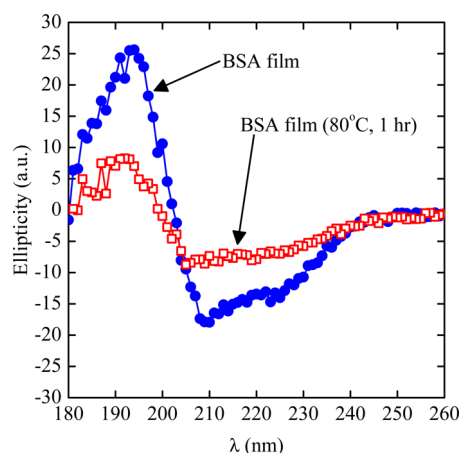

Figure 2. CD spectra of a protein thin film composed of bovine serum albumin (BSA) before (closed circles) and after (open squares) heat treatment.

comprises the dominant secondary structure of BSA.[30,31] The CD signal is substantially attenuated by heating the film (80 °C, 1 h) indicating, as expected, denaturation of constituent proteins at elevated temperatures (≥70 °C). The films that we examine in this study are not denatured, as confirmed via CD.

We measured the thermal conductivity of the protein films with TDTR.[32] Detailed specifics of TDTR and data analyses can be found elsewhere.[33−35] In brief, TDTR is a pump−probe measurement technique that utilizes the change in the thermoreflectance response of a thin metal film[36−38] in the time domain to determine the thermal transport properties of materials adjacent to the metal film. The train of pulses emanating from a short-pulsed Ti:sapphire oscillator are energetically split into the pump-and-probe paths. When immediately ejected from the oscillator, the laser pulses are centered at 800 nm with 11 nm of bandwidth and are ∼90 fs in duration. The pump path is modulated at 11.39 MHz, then focused to the sample surface generating an oscillatory temperature rise in the Al film at the modulation frequency. The probe pulses are then directed down a mechanical delay stage, focused onto the sample concentric with the pump pulses and back-reflected to a photodiode to monitor the change in reflectance of the probe pulses at the modulation frequency of the pump pulses. At the sample surface, the pump-and-probe pulse widths are unintentionally stretched to ∼400 and ∼200 fs, respectively. The $1/e^2$ radii of the pump-and-probe pulses on the sample surface were 25 and 6.5 μm, respectively, and we restricted the total laser power incident on the Al surface to a maximum of 25 mW, depending on the sample and temperature. We use lock-in techniques to monitor the small ($\sim10^{-4}$ to 10^{-5}) thermoreflectance signal.[39] The monitored data is related to the temperature change of the metal transducer, which at different pump−probe delay times is related to the thermal properties of the Al film and the materials underneath.[33−35]

All of the samples were coated with a nominally 90 nm Al film to facilitate TDTR measurements. Because TDTR analyses are sensitive to the thickness of this Al film transducer, we measured the film thickness of the Al during each measurement with picosecond acoustics.[40,41] The proteins films were sufficiently thick (≫1 μm) so that at the 11.39 MHz modulation frequency of the pump pulses the TDTR response

was completely insensitive to the thermal properties of the substrate. At this pump frequency, we estimate that our measurements sample ∼50−100 nm beneath the Al/protein interface. Therefore, the TDTR data were analyzed with a two-layer model of an Al film (the thickness of which was determined from picosecond acoustics) on a semi-infinite protein layer. In addition, because of the combination of the pump-and-probe spot sizes and the pump modulation frequency, the heat transfer is nearly entirely 1-D in the cross-plane direction.[35] We determined the thermal conductivity of protein films by fitting the ratio of the in-phase to out-of-phase lock-in output to a two-layer axially symmetric thermal model that accounts for pulse accumulation during the experiment.[33−35] We fit the thermal model to the data by varying the thermal conductivity of the protein. We assume literature values for the heat capacity of Al[42] and the heat capacity of the protein.[43,44] We deduce the thermal conductivity of the Al film via the Wiedemann−Franz Law and electrical resistivity measurements. Because of the low thermal conductivity of the proteins, our measurements were completely insensitive to the Al/protein thermal boundary conductance. Therefore, in our analysis, the only fitting parameter was the protein thermal conductivity. We took five to seven TDTR scans at different locations on four different samples of BSA (two on silicon and two on cover glass) and two different samples of myoglobin (both on silicon). The uncertainty in our measurements is determined by considering the repeatability of the measurements at the different sample locations, uncertainty in the local Al film thickness determined by picosecond ultrasonics, uncertainty in the thermal conductivity of the Al film, and uncertainty in the heat capacities of the proteins. In general, the uncertainty in the measured thermal conductivity of the protein films determined from our TDTR measurements is ∼15%.

The room-temperature thermal conductivities of our BSA and myoglobin films are 0.231 ± 0.031 and 0.190 ± 0.024 W m⁻¹ K⁻¹, respectively. The thermal conductivities of the BSA samples on silicon and glass substrates did not exhibit any difference within the experimental uncertainty. Our BSA measurements agree with previous measurement by Park et al.,[24] who determined the thermal conductivity of BSA as 0.265 ± 0.08 W m⁻¹ K⁻¹ from various measurements of BSA in water solutions.

Unlike measurements of the thermal conductivity of proteins in solution, our solid protein films allow us to measure the thermal conductivity of the protein structure at different temperatures without having to account for phase changes in the solution (e.g., freezing of water). We mount our protein samples in a liquid-nitrogen-cooled cryostat with optical access for our laser and measure the thermal conductivities of myoglobin and BSA from 77 K to room temperature. We only tested the thermal conductivities of the samples on silicon substrates to minimize steady-state heating from the absorbed laser power.[33] For the samples on the silicon substrates, we estimate that the steady-state heating from the absorbed laser will increase the sample temperature by <1 K over all temperatures. However, for the samples on glass substrates that we tested only at room temperature, we reduced the incident power but still approximate the steady state temperature rise as ∼20 K. As previously noted, we did not observe any difference in measured thermal conductivity so assert that this steady-state laser heating difference in the samples on different substrates did not change the thermal state of the

material. We expect additional heating in the protein samples by the absorbed energy from the modulated pump pulses at 11.39 MHz. This is the average heating in the volume sampled by TDTR (∼50−100 nm beneath the Al/protein interface).[33] This estimated temperature rise is <1 K for all temperatures and therefore negligibly affects our reported values.

The protein thermal conductivity as a function of temperature is shown in Figure 3. The thermal conductivity increases
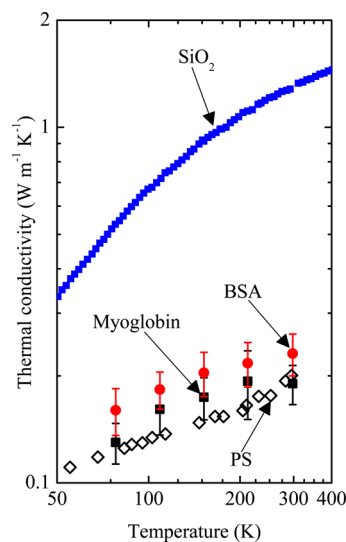

Figure 3. Thermal conductivity of BSA and myoglobin (this work), SiO₂,[47] and polystyrene (PS)[45] as a function of temperature. The increase in thermal conductivity in these systems over this temperature range can be ascribed to anharmonic vibrational interactions.

with temperature, although the values begin to level off upon approaching room temperature. We do not heat the sample substantially above room temperature to ensure that we are not denaturing the proteins. For comparison, we also show the thermal conductivity of polystyrene (PS),[45] which has been previously analyzed in terms of anharmonic coupling of vibrations.[46] The PS data show very similar trends to our protein data, which suggest that anharmonic coupling of vibrations in the protein structure is contributing to thermal conductivity. As another comparison, we show the thermal conductivity of SiO₂.[47] This increase in thermal conductivity has also been analyzed in terms of anharmonic coupling of localized vibrations.[46] In other words, much like SiO₂ and PS, anharmonic vibrational coupling contributes to the thermal conductivity of proteins.

In addition to demonstrating thermal conductivity measurements of solid proteins over a range of temperatures, one of our goals of this work is to lend insight into previous theories on fracton transport. The various fracton theories presented by Orbach and colleagues[19−22] claim that an increase in thermal conductivity over this temperature range is partially due to anharmonic fracton hopping.[20] However, the various amorphous materials that have been analyzed in terms of this fracton theory are not necessarily fractal, and they can also be well-described by other theories such as the minimum limit to thermal conductivity.[7−11] In fact, Freeman and Anderson[48] discuss that it is not clear what excitations are responsible for thermal transport in amorphous structures and Cahill and Pohl[49] conclude that the fracton theory does not apply to purely amorphous solids and polymers. Therefore, it is difficult

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to draw any conclusions about the validity of the fracton theories based on previous experiments. However, protein molecules are well-known fractal structures, and our data show qualitatively that anharmonic coupling of vibrations in this fractal network contribute to thermal conductivity. We cannot conclude whether the thermal vibrations in these solid protein samples are indeed fractons or simply localized vibrations; however, the observation of anharmonic-like trends in the thermal conductivity of these fractal structure will lend insight into future works.

To further the knowledge of how vibrations are contributing to heat transport in fractal proteins, we compare our measured thermal conductivity of myoglobin to the molecular dynamics simulations from Yu and Leitner.[13,18] In Figure 4, we plot the
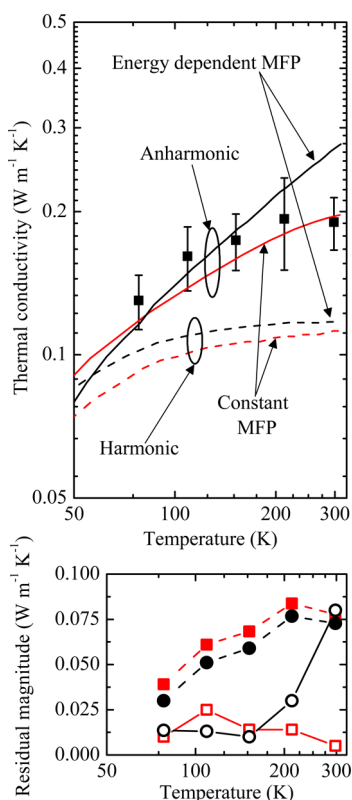

Figure 4. (top) Thermal conductivity of myoglobin (squares) as a function of temperature compared with the various models reported by Yu and Leitner.[13,18] The increase in the thermal conductivity with temperature indicates that anharmonic interactions are contributing to the thermal conductivity. Furthermore, on the basis of Yu and Leitner's computational findings, the observed plateau in the data at higher temperatures supports the fact that the mean free paths of the vibrations in proteins are localized to a common length scale. (bottom) Residuals between measured experimental data and the four models shown in the top plot (filled squares: harmonic and constant MFP; filled circles: harmonic and energy-dependent MFP; open squares: anharmonic and constant MFP; open circles: anharmonic and energy-dependent MFP).

myoglobin data from Figure 3 along with the results from four different simulations from Yu and Leitner accounting for: (i) harmonic interactions only with a constant mean free path (i.e., gray model); (ii) harmonic interactions only with a spectrally dependent mean free path; (iii) harmonic and anharmonic interactions with a gray model; and (iv) harmonic and anharmonic interactions with spectrally dependent mean free

paths. Over the temperature range investigated in our study, if anharmonicity was nonexistent, the thermal conductivity would not change with temperature according to Yu and Leitner's results and previous simulations by Shenogin et al.[13,18,46] As already established, the increase we observe in thermal conductivity is attributed to anharmonic interactions of the vibrations in the solid, as further supported by the agreement between our experimental data and the anharmonic models by Yu and Leitner. (The magnitude of the residuals between our data and the various models is plotted in the bottom panel of Figure 4.) Because of the uncertainty in our data and our high-temperature limitations to ensure our proteins are not denatured, we can not conclusively determine whether our data shows better agreement with the gray model[18] from Yu and Leitner than the spectral model.[13] However, this same temperature trend is observed in systems in which anharmonically interacting vibrations are localized to a single mean free path on the order of the intermolecular spacings, such as PS and SiO$_2$ (ref 46). More research must be conducted to determine the length scale of the localization of vibrations in fractal proteins and whether these vibrations are indeed a fracton.

The results and discussion above lend further insight into how anharmonic coupling of localized vibrations contribute to thermal conductivity. According to existing theories of thermal conductivity that consider anharmonicity as an independent scattering mechanism as opposed to an independent conduction channel, anharmonic scattering of vibrations (phonons) leads to a decrease in thermal conductivity.[50] Contrary to this prediction, in the limit of atomic disorder, it has been shown computationally that three-body interactions increase the thermal conductivity of the vibrational system.[51,52] These results have been explained in terms of the anharmonic decay of localized modes in disordered systems, which would otherwise not diffuse thermally.[13,18,20,51,52] Furthermore, Leitner et al.[53] theoretically estimated the vibrational energy transfer of normal modes, localized and extended, in a 1-D disordered glass finding that the anharmonic contribution to thermal conductivity is provided almost in full by spatially overlapping localized modes. This result is applicable to the protein structure, a randomly arranged heteropolymer structured by bonds of wide-ranging strength,[53] where most normal modes of protein and its secondary structure have shown to be spatially localized.[54]

In summary, we measured the thermal conductivity of solid, water-insoluble protein films of BSA and myoglobin from 77 to 296 K. The thermal conductivities of the proteins increase with increasing temperature. These increasing temperature trends indicate that anharmonic coupling of vibrations in the protein is contributing to thermal conductivity. This is consistent with vibrational transport in nonfractal amorphous solids, such as SiO$_2$ and PS. We cannot conclude whether the thermal vibrations in these solid protein samples are indeed fractons or simply localized vibrations; however, the observation of anharmonic-like trends in the thermal conductivity of a fractal structure will lend insight into future works and could form the basis for future validation of seminal theories from Orbach and colleagues.[19−22] Future computational research could elucidate the length scale of the anharmonic vibrational localization length in fractal proteins and whether this vibration is indeed a fracton.

Finally, future studies on the heat transport characteristics in solid protein films offer the potential to experimentally realize

novel phenomena in thermal conduction. For example, as pointed out by Yu and Leitner,[17] heat diffusion in a protein is subdiffusive. Therefore, conductance measurements of protein films of various thicknesses could give experimental insight into the mechanisms of anomalous heat diffusion.[55] Additionally, proteins are complex network structures. Liu et al.[56] have reported that thermal transport in complex network structures can give rise to asymmetrical thermal conductivity and non-Gaussian heat current distributions. Therefore, solid protein films could provide an avenue to realize these nontraditional regimes of thermal transport.

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Notes

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