

Excited-State Lifetimes of Far-Infrared Collective Modes in Proteins

Aihua Xie

Department of Physics, Oklahoma State University, Stillwater, Oklahoma 74078

Alexander F.G. van der Meer

FOM-Institute for Plasma Physics, 3439 MN Nieuwegein, The Netherlands

Robert H. Austin

Department of Physics, Princeton University, Princeton, New Jersey 08544

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Vibrational excitations of low frequency collective modes are essential for functionally important conformational transitions in proteins. Here we report the first direct measurement on the lifetime of vibrational excitations of the collective modes at $87\ \mu\text{m}$ ($115\ \text{cm}^{-1}$) in bacteriorhodopsin, a transmembrane protein. The data show that these modes have extremely long lifetime of vibrational excitations, over 500 ps, accommodating 1500 vibrations. We suggest that there is a connection between this relatively slow anharmonic relaxation rate of approximately $10^9\ \text{sec}^{-1}$ and the similar observed rate of conformational transitions in proteins, which require multilevel vibrational excitations.

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Vibrational motions in proteins are basic motions for protein dynamics and structural transitions. There are thousands of vibrational modes even in a small protein [the number of vibrational modes = $3 \times (\text{number of atoms} - 6)$]. One of the central problems in the dynamics of protein action is determining the lifetimes of the low frequency collective modes of a protein molecule. The importance of these collective mode dynamics comes from two fundamental aspects of protein action. First, proteins have close-packed structures, and changes in the conformation of a protein require the collective motion of a large fraction of the protein's atoms. Second, to make a conformational transition from one structure to another the corresponding collective modes (Fig. 1) must be excited up the vibrational ladder in order to cross the transitional energy barrier. The excitation and relaxation of these collective modes are generally achieved via anharmonic interactions with other normal modes for energy exchange. It is believed that low frequency collective modes are responsible for the directed flow of conformational energy for a variety of vital biological processes ranging from electron transfer [1–3] to enzyme action [4,5]. However, without the knowledge of the actual lifetimes of these states it is extremely difficult to connect theory and experiment.

The frequencies of the vibrational modes in a protein are broadly distributed from approximately $3700\ \text{cm}^{-1}$ (O-H stretching) to a few cm^{-1} (collective modes of the entire protein) in the mid- to far-infrared regions. Strong force constants and small masses give rise to high vibrational frequencies, such as O-H, N-H, and C-H stretching. Low frequency modes may be localized with weak force constants, such as twisting motions ($\sim 120\ \text{cm}^{-1}$) of methyl groups, or global collective motions with a large number of atoms. The concerted motions of a large number of

atoms at low frequencies appear to be mainly bending, deformation, and twisting motions involving changes in bond angles. Both molecular dynamics simulations [6] and preliminary studies based on *ab initio* density function theory [7] show that approximately 15% of all vibrational modes are below $200\ \text{cm}^{-1}$, giving rise to a broad band of far infrared (FIR) absorbance centered at about $100\ \text{cm}^{-1}$ as we show in Fig. 2 for the protein studied here, bacteriorhodopsin. Since localized twisting motions of nonpolar groups show very weak infrared absorption, we estimate

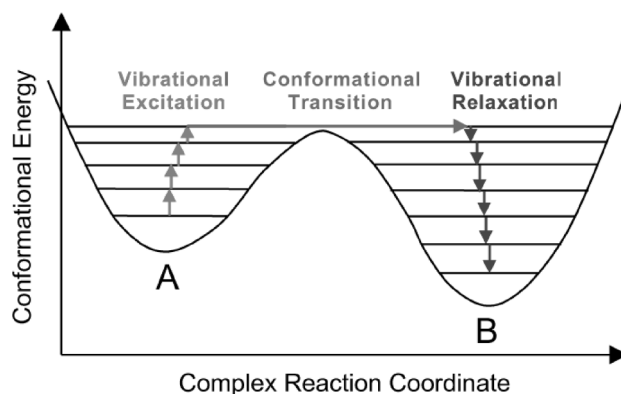


FIG. 1. A scheme depicting multivibrational excitations of a collective mode up the energy ladder for conformational transition. Two energy wells along a reaction coordinate are shown. The horizontal lines indicate the energy levels of vibrational ground state (the lowest) and excited states. The nonequal separation between two adjunct energy levels illustrate the anharmonic nature of the collective mode. In order for the protein to change conformation from structure A to B, it is necessary for the protein to climb up the vibrational energy ladders until it is possible to cross the transition state (the peak between A and B). The energy required for vibrational excitation in this mode may be obtained via anharmonic interaction with other vibrational modes (intramolecular energy transfer) or via anharmonic interaction with solvents (intermolecular energy transfer).

that the FIR absorbance band of bR is due to at least 800 low frequency modes which are collective modes involving many amino acid residues.

Because of the dense manifold of states in the FIR and the possibly short lifetime of these modes, the FIR region does not show a spectrum of relatively sharp spectral features as does the mid-IR but instead a broad continuum of states. Since there are no obvious sharp spectral features, it is tempting to use the fundamental relationship $\tau\Delta\omega \sim 1$ connecting lifetime τ and bandwidth $\Delta\omega$ of a transition to predict that the lifetime of the collective modes must be on the order of subpicoseconds. However, in a complex molecule like a protein with its many conformational sub-states [8] the heterogenous line broadening is such that it is impossible using absorption spectroscopy to determine mode lifetimes since the homogenous linewidth $\Delta\omega$ is not known.

Mid-IR pump-probe studies of vibrational relaxation of the amide-I excitation indicate that energy transfer out of the modes at $6\text{ }\mu\text{m}$ occurs on the picosecond time scale [9–11], but we do not know the path along which the energy flows nor have there been measurements of the cooling times of the collective FIR modes which are presumably the penultimate repositories of excess energy in a protein prior to solvent interactions. Pump-probe measurements which can directly measure relaxation processes in the region of $100\text{ }\mu\text{m}$ (100 cm^{-1}) are difficult due to a lack of intense picosecond FIR sources. The FELIX free electron laser is one of the few sources capable of delivering $10\text{ }\mu\text{J}$ pulses in 10 ps in the FIR. These experiments are challenging even with a source such as FELIX because the oscillator strength of the FIR transitions is quite weak and there is considerable water absorption in this region, so thin hydrated films of the protein must be used and sophisticated common mode noise cancellation schemes must be used to obtain the necessary sen-

sitivity to 10^{-3} absorbance changes. Figure 3 shows the picosecond pump/probe apparatus we have constructed to obtain the requisite sensitivities, using a delayed reference pulse scheme [11] in conjunction with a 1 GHz bandwidth liquid helium cooled gallium-doped germanium detector to do these experiments.

We measured the relaxation rate of excited collective modes in proteins using time-resolved picosecond far-infrared pump/probe. Bacteriorhodopsin, a well studied membrane protein containing seven transmembrane alpha helices, was employed in this study because it is a protein which undergoes rapid biologically important conformational transitions [12,13]. Protein samples were made on $400\text{ }\mu\text{m}$ thick films of polyethylene, a polymer which is transparent at $87\text{ }\mu\text{m}$. Film integrity and adhesion to the polyethylene was also enhanced if the protein contained 10% glycerol. We found it important to maintain the thin films at a hydration level of 90% for two reasons: (1) films that were dried to 20% hydration did not yield large pump/probe signals and (2) dry films cracked and flaked off the polyethylene. Important issues pertaining to the role of viscosity and hydration on the relaxation times of the FIR collective modes need to be explored but are not addressed in this preliminary communication because of difficulty in achieving transmission of the FIR through a water film.

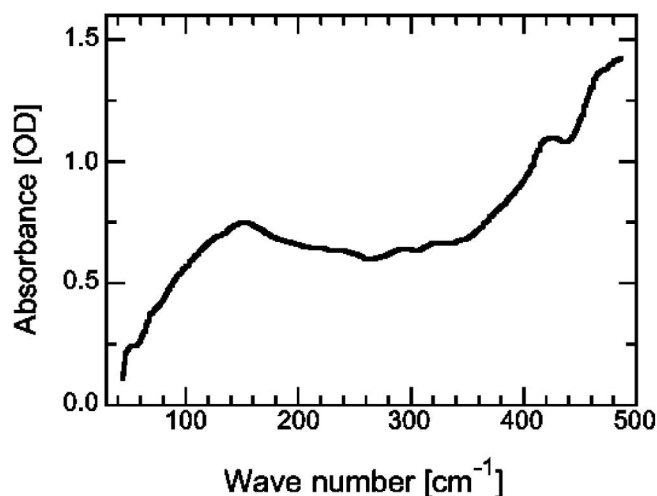


FIG. 2. The FIR spectrum of a thin film of bacteriorhodopsin. Approximately $5\text{ }\mu\text{L}$ of a 10 mg/ml solution of a suspension of bR was dried in a 90% relative humidity atmosphere to a spot of about 1 cm diameter.

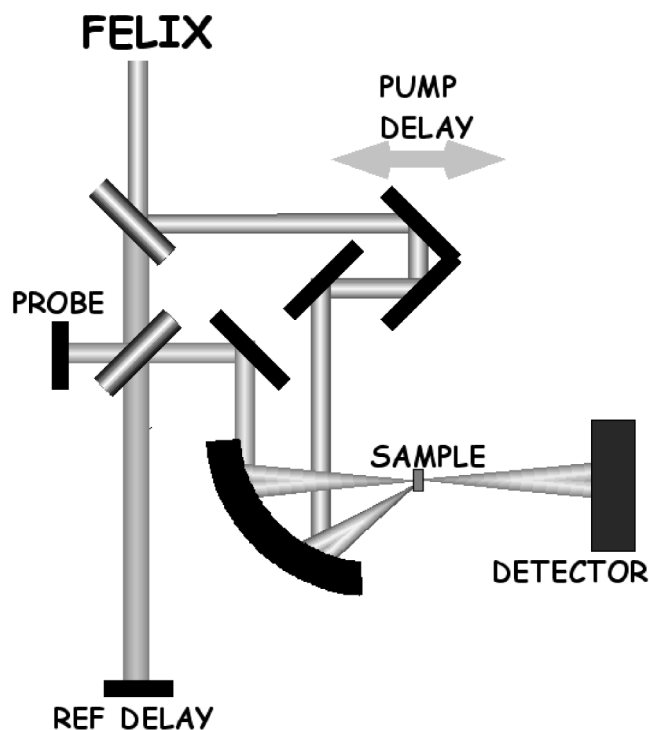


FIG. 3. The pump-probe apparatus constructed at FELIX. Details of the design of this experiment can be found in [11]. Maximum times that could be observed were limited by the length of the delay line used (500 ps). The heterodyne mixing electric field pattern of the picosecond pulses from FELIX reveals the 10 ps pulse expected from a 76 period wiggler operated at $87\text{ }\mu\text{m}$.

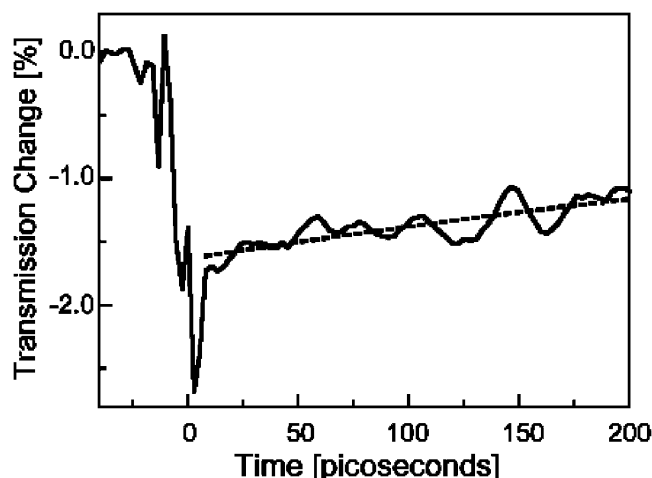


FIG. 4. Pump-probe response of bR at $87 \mu\text{m}$. The data are shown as the percentage transmission change. The spot size of the pump beam was $1000 \mu\text{m}$ and the pump energy per micropulse was $1 \mu\text{J}$. The dotted line is a single exponential fit to the data, with a time constant of $500 \pm 100 \text{ ps}$.

We were restricted from physical optics considerations in the FIR to using 1 cm input beam diameters D to our off-axis focusing parabolas and focal lengths f of our focusing off-axis parabolas of 12 cm , so the spot sizes obtained were on the order of $1000 \mu\text{m}$. This combination of relatively long pulses (10 ps) and large spot sizes ($1000 \mu\text{m}$) means that a $1 \mu\text{J}/10 \text{ ps}$ pulse produces a peak FIR intensity of $10^{11} \text{ watts/m}^2$. Although the peak power is very high, since our sample was $20 \mu\text{m}$ thick and our focal spot 10^{-3} m in diameter the sample temperature rise per micropulse at the focus was 10^{-3} K or 10^{-1} K in a 100 micropulse wide macropulse. This is far smaller than the 50°C temperature rise experienced at $6 \mu\text{m}$ [14] so local heating concerns were not important in the FIR.

Figure 4 shows the pump/probe signal observed for hydrated bacteriorhodopsin films. As discussed above, it was important for the film to be hydrated, dried films at a relative humidity of 50% did not show a pump/probe signal at the 0.1% transmission level. The FIR pump/probe signal shows a 2% (transmission) bleaching signal. The fast oscillations observed within the rise time of the bacteriorhodopsin signal are heterodyne mixing artifacts due to the strong scattering of the bR containing vesicles within the sample and do not represent actual dynamic effects. Curve fits shown in Fig. 4 to the bR signal give a decay time of $500 \pm 100 \text{ ps}$, and are far longer than observed in the mid-infrared region [11]. The longest delay time measured was limited by delay line travel. The most striking thing about these pump-probe signals is the apparently

extremely long lifetime of the collective modes compared to lifetimes typically observed at the shorter wavelengths mentioned above or predicted from the FIR absorption linewidth discussed above.

It is informative to examine a form of scaled lifetime of our data; that is, the number of cycles before the energy is transferred out of the mode. This is a more robust indication of the anharmonic mode coupling strength α . It is also important to stress that pump/probe signals measure only the longitudinal relaxation times T_1 of the transition, not the dephasing times T_2 [15], so these cycles are not necessarily phase coherent over the duration of the relaxation process.

Table I reveals that the excited FIR modes takes more than 1000 vibrations prior to vibrational relaxation, whereas the excited amide I modes takes 80 vibrations. The remarkably long lifetime of FIR vibrational excitation compared with the amide I mode may be related to fewer pathways for further energy flow in the FIR and relatively weak solvent damping with low hydration. There have been many attempts to model the relaxation dynamics of the collective modes of a protein using molecular dynamics starting with the work of Go *et al.* [16] and Brooks and Karplus *et al.* [17]. Although it is possible to enumerate a great number of normal modes in the harmonic approximation which would indicate that there are many degrees of freedom into which a given mode can decay, in reality the coupling of these modes within the protein and to the solvent is a quantum mechanical problem and the classical trajectories and potential surfaces used in Newtonian mechanics based calculations can be misleading. Leitner and Wolynes [18] have discussed this issue of the restriction of available modes using the model of local random matrix theory and a related recent study by Moritsugu *et al.* [6] uses the time-dependent Fermi's golden rule formalism for a system crossing to show that a given mode q_i interacts only with a small subset of the modes that are selected by overlapping frequencies (Fermi energy resonance).

In addition to energy conservation, an equally important aspect to collective mode relaxation comes from the extent of the spatial overlap of the mode energy transfer rate which comes from the anharmonic third-order spatial overlap in the normal mode nonlinear energy term $\alpha q_i^2 q_j$ where q_j is the spatial coordinate of the j th mode degenerate in energy with the i th mode and α is the nonlinear coefficient of the potential surface which couples the modes together. Even within the classical molecular dynamics simulations there is not a common opinion about the influence of the solvent on the damping times [19], ignoring

TABLE I. Cycle lifetimes.

Transition	Energy scale	Frequency	Lifetime	Cycles
Vibrational (amide I)	0.2 eV	$4 \times 10^{13} \text{ Hz}$	2 ps	80
Collective modes ($100 \mu\text{m}$)	12 meV	$3 \times 10^{12} \text{ Hz}$	500 ps	1500

other issues concerning the influence of sampling times on the long time dynamics [20]. Normal mode dynamics and relaxation calculations by Levitt *et al.* [21] found that the low frequency collective modes were not strongly damped by the solvent because the amplitude of the modes was only 0.3 Å, less than the diameter of a water molecule and making macroscopic based hydrodynamic approximations questionable, while Kitao *et al.* [22] found that the modes basically damped within one period of oscillation, that is, at the transform limited discussed above. The only experimental tests we have found concerning normal mode lifetimes comes from studies of FIR emission of proteins when optically excited at optical wavelengths [23] and elastic scattering of neutrons from the collective boson peak seen below 100 cm⁻¹ [24].

Figure 1 was an attempt to show how vibrational excitation of the reactive vibrational mode is a basic requisite for structural transition in a molecule (such as proteins) and its relation with transition rate. Initially the molecule is in an equilibrium structure with the reactive mode in position q_A . In order for the molecule to assume a new structure with the reactive mode in position q_B , the reactive mode must be sufficiently excited to the energy level which is close to or higher than the transition energy barrier. With conservation of energy, the processes of these multivibrational excitations require vibrational energy exchange with other vibrational modes of the molecule and/or via collisions with solvent molecules. The time needed to reach the energy level for structural transition is related to the vibrational relaxation time, since the shorter the vibrational relaxation, the stronger the vibrational couplings, and the faster the energy flow (in and out) of the reactive vibrational mode. Once the reactive mode reaches the energy level for structural transition, the long relaxation time may indicate that it takes much shuttling between the old position q_A and the new position q_B before eventual structural transition (or returning to the old structure). Therefore, the rate of structural transition is closely related to the vibrational relaxation time of FIR collective modes. It is intriguing and yet challenging to develop a fundamental rate theory that quantitatively links the vibrational relaxation rate with the rate of structural transitions.

Our results indicate that even though these proteins are hydrated the coupling strength of the collective modes to the solvent is relatively weak and the homogenous modes within the heterogenous envelope are relatively underdamped. This has important implications for the fundamental physics of energy relaxation rates in biomolecules and the collective molecular dynamics that these remarkable molecules can utilize.

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