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Hidden harmonic quantum states in proteins: Did Davydov get the sign wrong?

Aihua Xie[1], Britta Redlich[2], Lex van der Meer[2], Saurabh Vyawahare[3] and Robert H Austin[3,4]

[1] Dept. of Physics, Oklahoma State University, Stillwater, OK 74078, USA
[2] Radboud University Nijmegen, 6500 GL Nijmege, The Netherlands
[3] Dept. of Physics, Princeton University, Princeton, NJ 08544, USA

E-mail: aihua.xie@okstate.edu, b.redlich@science.ru.nl, l.vandermeer@science.ru.nl, saurabhv@princeton.edu and austin@princeton.edu

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Abstract

We revisit mid-IR pump–probe experiments at the FELIX picosecond Free Electron Laser which probed the vibrational dynamics of the α-helix rich protein in search of long-lived anharmonic trapped vibrational states (solitons). We analyze and try to understand something puzzling that we observed in the context of unusual 'hidden' quantum phenomena in proteins which probably are of no biological consequences, but bears re-examination. We observed in a narrow (0.5 cm^{-1}) spectral range of the amide-I band a very large response in terms of degenerate 4-wave mixing scattering. We propose that this narrow but strong scattering signal is not due to anharmonic trapping but rather is the imaginary (index of refraction) component of a super-linear response of the amide I band to high levels of vibrational excitation.

Keywords: solitons, proteins, hamonic, spectral width

(Some figures may appear in colour only in the online journal)

1. Introduction

One of the fundamental mysteries in biological physics is the coupling in proteins between ATP hydrolysis and the directed action of proteins either on the breaking of specific bonds or to the physical translocation of the protein itself on a substrate, such as the movement of myosin on actin which results in muscle contraction [1]. There have been enormous strides in *measuring* the discrete step-like motions of motor proteins using single-molecule techniques with astonishing nanometer precision using optical wavelength light [2, 3] but what still remains beyond reach is a physical understanding of these energetic events: exactly how, at the atomic scale, do these fantastic nanomachines work?

There has long been the belief that understanding these dynamics at a deep and fundamental level must include a quantum mechanical understanding of the process, not a classical Newtonian approach. A fair amount of time has been spent hunting down one prospective quantum mechanical model, namely the Davydov soliton along the α-helix backbone of the protein [4–6]. These experiments were challenging; we used a tunable ps mid-IR Free Electron Laser to try and observe the long-term (microsecond or greater) trapping of coherent excitation in proteins which had been proposed by several theorists [7, 8].

These experiments were successful in the sense that we directly observed vibrational excited state population relaxation on the picosecond time scale, and transfer of coherent excitation into the incoherent thermal bath: but we we did not see the trapping on the microsecond time scale of short (ps) coherent light pulses in the amide I band of a generic alpha-

[4] Author to whom any correspondence should be addressed.

helix rich protein, myoglobin [9]. At the time we wrote that 'final' paper we believed that we had convincingly shown that the Davydov self-trapping mechanism was not present at least in the simple protein, myoglobin, and unlikely to be present. We would like to re-think that paper and suggest that it might have a different story to tell.

2. The conformational distribution of proteins

One fundamental misconception about proteins is the belief they fold to a unique ground state, and in that sense represent a crystalline solid. In fact, they are more like a glass than a crystal. It is important to understand that proteins do not fold into a single, well defined and unique conformation. This was brought out by a simple experiment involving the observation of a chemical reaction in myoglobin at cryogenic temperatures, from 300 to 4.2 K [10]. It was observed that below 200 K in a glycerol water glass the rate of the reaction, the recombination of carbon monoxide with the iron ion in the heme group of myoglobin could not be characterized by a single rate constant but instead seemed to be due to a *spectrum* of rates. A simple question: Are the data fit by a sum of multiple but discrete exponentials? About 6 exponentials would be needed to fit the data, expanded data by Frauenfelder's group which covers 12 decades of time would need on the order of 12 exponentials, so as the number of exponentials goes to infinity it becomes clear that a continuous distribution of states exists. We thus assume that the activation energies for recombination are given by a probability distribution $g(E)$ for finding a molecule with activation energy E, or equivalently a distribution $g(k)$ for finding a molecule with rebinding rate constant k. The kinetics then become:

$$N(t) = N(o) \int g(k) \mathrm{e}^{-kt} \mathrm{d}k. \tag{1}$$

Basically, this experiment revealed that at low temperatures the protein seemed to have a time-invariant and temperature invariant *spectrum* of activation energies. Other workers have proposed that the distribution of rates is due to internal dynamics within a single molecule [11] but several experiments have ruled that possibility out [10]. In this kind of inhomogenous kinetics it is assumed that what would appear to be a homogenous system (a protein folded into a unique ground state with a single reaction rate k_o is incorrect: proteins fold into a distribution of ground states with different reaction rates for different conformations.

3. Mid-IR dynamics on the picosecond time scale after coherent excitation

Unfortunately we do not know the mapping between the conformational spectrum of the protein myoglobin (Mb) and the line width of the amide-I mid-IR band of the protein at about 6 μm. The amide-I band is where it has been proposed that quantum mechanical energy trapping can occur. It is possible, but unlikely, that the conformations (which give rise to the distribution of recombination barrier heights), and are 'frozen in' at temperatures below about 160 K, play no role in line width of the amide I bands in the IR spectrum of myoglobin. If the amide I band is homogeneously broadened then the observed linewidth of the amide-I band in myoglobin, approximately 30 cm^{-1} at 290 K, implies a longitudinal relaxation time T_1 of approximately 10^{-12} s (1 ps). This very short relaxation time would indicate, as we have stated before, that there is no long-lived self-trapped energy state in proteins via the Davydov mechanism of any biological interest.

However, it would seem that the mid-IR amide I band is not homogeneously broadened because of the unusual temperature dependence of the amide-I band, as was discussed in a previous paper [12], we refer the reader to that paper for details. The amide I band of Mb is inhomogeneously broadened in a curious way: on the blue (short wavelength) side of the amide I band there is very little temperature dependence of the absorbance of the amide-I band, while the red (long wavelength side) shows a strong temperature dependence. One interpretation of this is that the inhomogenous broadening consists of two parts: (1) a harmonic response core centered at about 6.05 μm, and on the red side a strongly temperature dependent region centered at approximately 6.17 μm which is anharmonic. This red-side band loses oscillator strength with increasing temperature due to a strong anharmonic coupling to the phonon bath of the solid matrix [12].

The expectation then is that under the intense pumping of the mid-IR band (about 10^{12} watts cm^{-2}) by the Free-Electron Laser used in these experiments at FELIX the rapid detuning of the narrow band pump pulse as the anharmonic vibrational ladder is climbed the result is that relatively small amounts of energy can be deposited in the red-shoulder due to rapid detuning and strong phonon coupling of the amide I band. The result is that in degenerate 4-wave mixing, which is responsible for the pump–probe signal, that only relatively small pump–probe signals can be obtained for strong phonon-coupled oscillators because of the rapid (picosecond time scale) flow of vibrational energy out of the anharmonic band [12].

All of this suggests, as we proposed in [9], that the original models of the Davydov solition were misguided, in that the temperature dependent band to the long wavelength (red) side of the amide I band is actually so strongly coupled mode to the phonon heat bath, it does not represent a quantum trapped state, and in fact little energy can be pumped into it. It acts exactly opposite to the way that was originally proposed by Davydov, as a strong dissipative damper of coherent energy trapping.

In spite of our failure to see any truly long-lived real (absorbing) coherent states on the long-wavelength side of the amide band (100s of picoseconds or longer) when pumped with high intensity short (ps or less pulse duration) as predicted by Davydov, surprisingly rather dramatic things happen when narrow band (long pulse, and hence lower intensity) excitation is done on the short wavelength side of the amide I band, where one would expect to see no signal at all. We have

shown in a paper [9] that this region can be pumped effectively by *widening* the temporal pulse width of the FEL and thereby *narrowing* the spectral width of the pulse. We refer the reader to [9] where the data is presented showing a very large (effectively 50%) transmission change at 6.05 μm, where one would expect to see NO pump–probe signal.

The micropulse width δt of the FELIX FEL could be varied between 0.5 and 10.0 ps, giving rise to a spectral pulse width δw (in cm^{-1}) by the fourier transform:

$$\delta w = \frac{1}{4\pi c \delta t}. \tag{2}$$

Thus, the line width of the FEL pulse could be varied between 5.5 and 0.25 cm^{-1}. The energy of the micropulse is independent of the width of the micropulse, hence the average intensity $\langle I(t)\rangle$ of the pulse should scale inversely with pulse width. If in the strongly anharmonically coupled regions energy flow out of the amide-I oscillators (measured by the longitudinal relaxation rate T_1 into the heat bath) on the picosecond time scale or less, it is difficult to accumulate a significant population in excited state levels with a pulse whose pulse width τ is greater than T_1. However, in a highly harmonic T_1 will be large relatively speaking and effective pumping into highly excited vibrational levels is possible even with relatively low intensity pump excitation. Hence, one would expect for very long dephasing times in a highly linear system that a very long pulse, of narrow spectral width, will simply accumulate excitation energy into successively higher vibrational levels even if the intensity is relatively low compared to the short pulse case, assuming that the linewidth of the linear coherent state is very narrow.

On the other hand, because the system is highly linear, there would seem to be no way to spectroscopically (using real excitation absorbance) determine the vibrational level of excitation, since each level is spectroscopically identical in a truly harmonic system and hence the real absorbance cannot change as a function of excited state levels within the vibrational manifold. In pump–probe measurements a common input beam of coherent laser light is split into an intense pump beam and a much weaker probe beam. The intense pump beam is delayed in time with respect to the probe beam via movable optical delay line, the two beams are focussed to a spot size Δd in the plane of the sample. The two beams are not collinear but instead intersect at an angle $\Delta\theta$. Since the two beams originate from the same pulse, they are degenerate in frequency, have the same pulse width δw and have a fixed phase difference at the peaks of the pump and probe pulse shapes depending on the distance separating the pump and probe beams. If the sample has an intensity dependence to the oscillator strength ϵ the mixing of the two beams at the sample will create modulations in the optical response of the system. However, because of the angular separation between the pump and probe pulses there is a running phase shift across the face of the pump beam due to pump–probe interference and a diffracting gradient can occur, which can coherently scatter the pump into the probe direction if the phases are remembered by long dephasing times. However, in the simplest case where the response of the system rapidly becomes incoherent (very short transverse dephasing times T_2), then the probe beam merely measures the real absorbance change of the sample due to the energy deposited in the sample incoherently.

In the case of a purely harmonic system there can be no real absorbance change as a function of excitation levels since all levels are equally spaced in energy and hence we might expect that there would be no change in the probe transmission as a function of pump-delay relative to the probe. However, the absorbance spectra of the amide I band represents the dissipative real part χ_R of the response of the system. There is a corresponding imaginary component χ_I (the index of refraction) which is the non-dissipative response of the system, and it is not directly measured in typical fourier-transform infrared spectroscopy, which is a linear process. Causality demands there be a connection between the real and imaginary parts of the response of a system which interacts with light, this correlation can be computed because of causality constraints by the Kramers–Kronig transform [13] (also know as the Hilbert transform). If one knows either the real or imaginary parts of the system response to an excitation $I(t)$, using the Kramers–Kronig transform it is possible to construct the corresponding response. Formally, the Kramers–Kronig transform between the real and imaginary parts of a system response are:

$$\chi_i = 1 + \frac{2}{\pi} P \int_0^\infty \frac{\omega' \chi_R(\omega')}{(\omega')^2 - \omega^2} \mathrm{d}\omega', \tag{3}$$

where P is the Cauchy principle value, a way to deal with the apparent singularity in the integral at $\omega = \omega'$. There are many numerical implementations of the Kramers–Kronig transform, in the work shown below we used the equivalent Hilbert transform in MatLab [14].

As an example of using the Kramers–Kronig transform to extract the complex response, we use the amide-I temperature dependent absorption spectrum of the amino acid serine as an example of the generality of the procedure, and the unusual nature of the amide I band absorbance, which is not confined to globular proteins. Figure 1(A) shows the temperature dependence of L-serine dissolved in 75% v/v d-glycerol/D2O. Rather like Mb, as shown in [9], most of the temperature dependence of the amide I band occurs on the long-wavelength (red) side of the amide I band, hence one would think that in a pump–probe experiment nonlinear effects should be seen to the red but not the blue side of the amide-I peak. Note that the imaginary response has both positive and negative values and the negative part is peaked to the short wavelength side of the maximum of the real response, while the positive part will be peaked to the long wavelength side of the real response. Since the positive part is positioned in the region where the real part is decreasing with increasing temperature, the two changes will tend to cancel each other out, while on the short wavelength side the there is almost no change in real absorbance with temperature, and so the negative imaginary index of refraction change is not cancelled out, and a maximal signal can be expected.

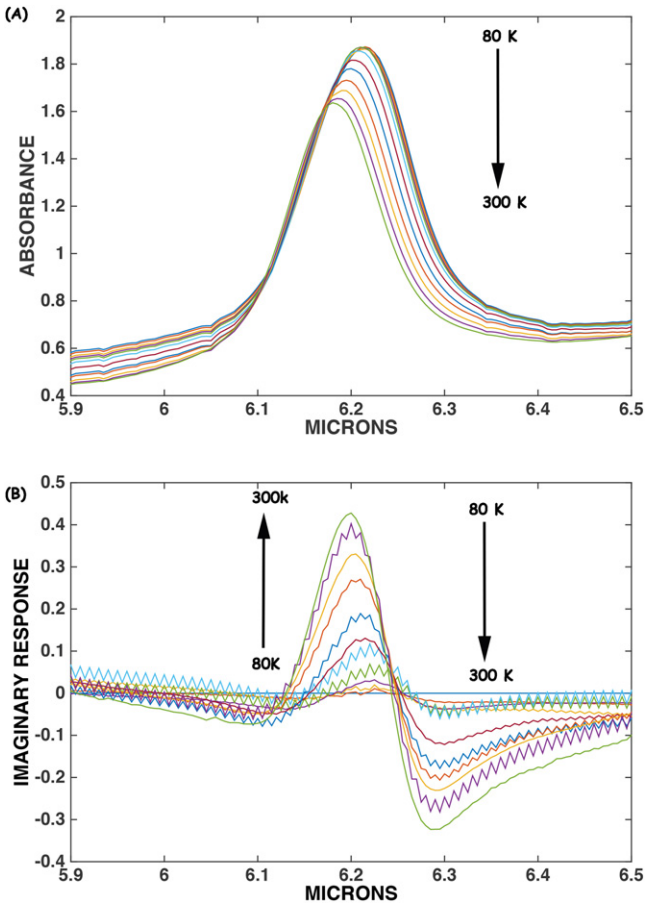

Figure 1. (A) The real (absorbance) temperature dependent spectrum of L-serine dissolved in 75% v/v d-glycerol/D2O. (B) The imaginary (refractive) temperature dependent response of L-serine as computed by a Hilbert transform in MatLab. The high-frequency wiggles are artifacts of the transform algorithm.

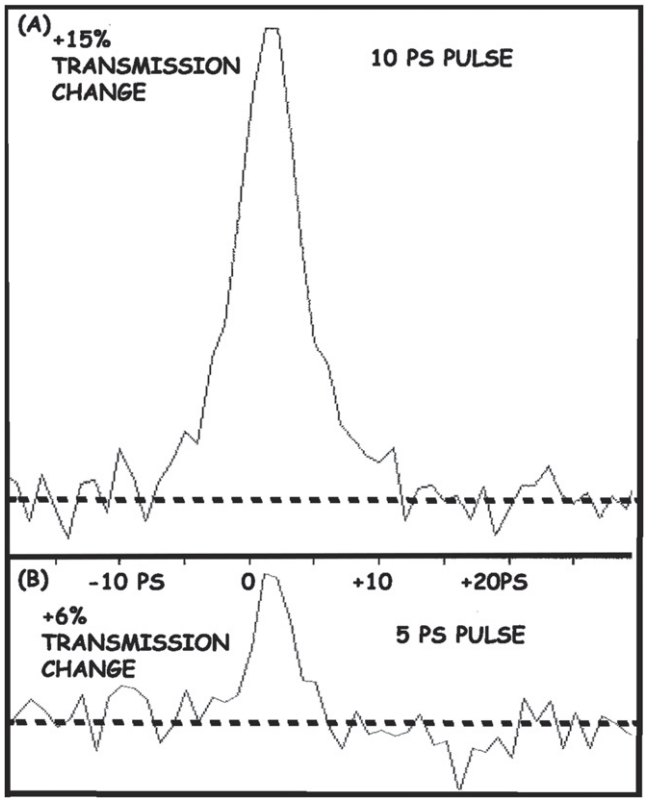

Figure 2. (A) Serine pump–probe response at 6.1 μm with a 10 ps FWHM pump. (B) Serine pump–probe response at 6.1 μm with a 5 ps FWHM pump.

The Kramers–Kronig transform to the L-serine absorbance spectrum reveals in figure 1(B) that as expected the imaginary response of the amide I band, that is, the index of refraction n, has a strong temperature dependence, and this temperature dependent grating should also scatter light coherently into the probe grating direction. If the two pulses do not overlap in time, then no grating is created and in terms of the probe beam there will be no memory of the previous presence of the pump beam, unlike in the absorbance mode where the temperature imprint of the pulse remains. In the imaginary part, scattering can only occur when the pump and probe interfere to create a grating.

The amplitude of the L-serine pump–probe signal when scanned across the absorbance spectrum with relatively narrow spectral width shows similar puzzling effects as does a Mb signal. Figure 2 shows a wavelength scan with a narrow (less than 1 cm^{-1}) scan across the amide-I band of L-serine of the pump–probe response. A large signal is seen with narrow spectral width signal (1 cm^{-1}), while a broader spectral pulse (1 cm^{-1}) yields a smaller effective transmission change.

The lack of a long-time rise in transmission after pulse if the two pulses do not overlap in time would indicate that for narrow spectral pulses that the scattering is predominately due to the complex response of the system, and not the real component. We would then propose that the large response seen in proteins on the blue side of the amide I band is due to a complex index of refraction grating driven by pumping highly linear modes of the amide I band of the protein, this complex grating (index of refraction) scatters the pump into the probe beam coherently. A further proof that this is a grating signal caused by the complex index of refraction changes mapped over from the long wavelength side of the amide I band and not a real population level change can be measured by rotating the pump polarization to be $\pi/2$ radians from the probe polarization: in that case the two beams cannot interfere and create a grating, and only anharmonic population changes can be observed. Figure 3 shows that this is indeed the case in the case of the protein myoglobin.

4. Conclusions

It would seem to be highly ironic that in searching for Davydov localized states, quantum mechanical localization driven by an anharmonic coupling, while we showed in [9] that they probably not exist, but here we at least point to the possibility rather there exists in at least amino acids and the protein myoglobin a highly linear band within the amide-I manifold which seem capable of being pumped to high vibrational levels, *because* of the high linearity of the potential function, not in spite of it. Conditioned to look at

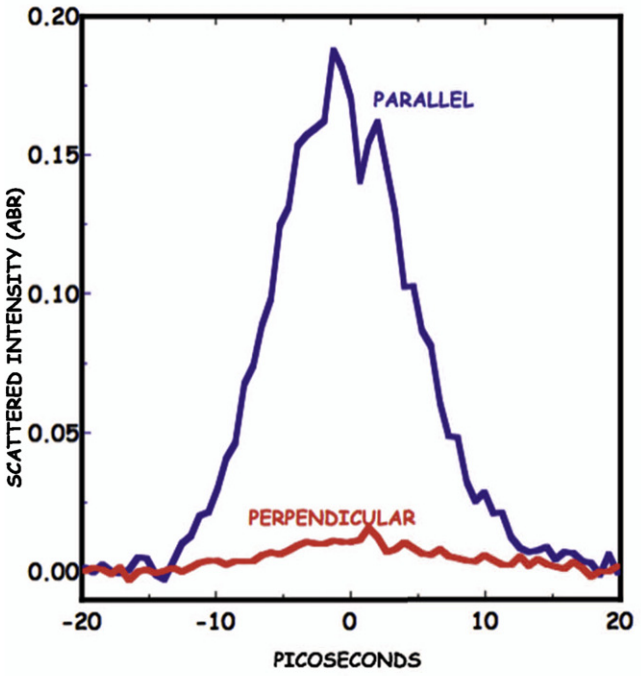

Figure 3. Dependence of the narrow band signal at 6.05 μm in Mb with the relative polarization of the pump and probe coherent pulses.

long excited state lifetimes and Rabi oscillations as we were, we missed the possible significance of this result. However, what we present here is unfortunately just some almost anecdotal evidence, by no means a detailed exploration of the phenomena and a full resolution of it.

There is a further fault to our experimental program as it was carried out. The issue here is not so much the trapping of coherent vibrational energy, it is the transport of energy. And it is not so much the trapping of coherent energy within a single protein, but rather the coherent transport of energy along a collection of connected protein molecules. The obvious candidate for this experiment are microtubules. Briefly, microtubules are part of the cytoskeleton, they form long polymer filaments made of the protein tubulin which can be on the order of 10 up to 50 μm long [15]. Theorists [16] concentrated on microtubules in their theories of possible links between quantum mechanics and neuronal computation because microtubules also exist of course in neurons, and the theorists need something to propagate the wave functions long distances without decoherence. Unfortunately, there are two basic divisions in almost any field of science: experiment and theory. In theory, often it is easy to think that all things are possible given a lucky combination of various parameters which may or may not be physically reasonable. It is then possible to develop a theory which unfortunately while mathematically correct may have no connection with reality. On the other hand in experiment, we can become so earth-bound due to a lack of imagination, that the experimenter can miss a really exciting piece of physics that unifies and explains some puzzling, profound effect.

The puzzling, profound question that we attempted to address in our experiments was: What is the fundamental way that chemical free energy could get possibly trapped in a protein rather than flowing incoherently and ergodically into the thermodynamic limit of equal occupation of all the degrees of freedom? Unfortunately we possibly stumbled at the end of the nearly 10 years of experiments on something very interesting in proteins indicating a truly coherent process in proteins but we did not continue the work on more interesting systems such as microtubules.

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