COHERENT PROTEIN DYNAMICS EXPLORED AT FELIX

R. Austin, Princeton University, Aihua Xie, Oklahoma State University
B. Redlich, Lex van der Meer, FOM Institute for Plasma Physics

Abstract

We have used extremely narrow (1-2 cm^{-1}), high energy pump-probe time domain spectroscopy to probe the heterogenously broadened amide I band in several proteins. We find that the short wavelength side of the amide I band can give rise to very large (50%) bleaching signals, indicating a very long dephasing time of at least 20 ps to the internal amino acid amide I stretch band.

INTRODUCTION

At a recent conference, we heard an eminent theorist refer to the "original sin" committed in a talk. The theoretical talk was about self-localization of vibrational (not electronic) energy and the generation of solitons in a biopolymer, in this case DNA. It was a good talk, very clear and carefully presented. What then was the sin? The critic was convinced that relevant biological motions in biology are all overdamped and therefore diffusive in nature. If they are overdamped, then (perhaps) issues of energy self-localization and solitons are simply irrelevant at least at the picosecond time scale. So, our critic's point was: if you don't address head-on issues of overdamping times in biological vibrational dynamics you do commit an original sin: everything after that is tainted.

There is an additional issue that wasn't mentioned in the charge of original sin, that of dephasing time. Using the language of NMR and the Bloch equations, there are two parameters that characterize an excited state: the longitudinal relaxation time T_1 which is a measure of the lifetime of an excited state, and the transverse relaxation time (pure dephasing time) T_2^*, which is a measure of how long the system remains in phase [1]. It could be, as we discuss below, that the dephasing time of any coherently excited vibrations can be much shorter than the excited state relaxation times and determine the linewidths of the transition. In that case, it is almost as great a sin to ignore a short T_2^* as ignoring T_1, for many non-linear effects need the coherence times of the excitations to be long as well to be effective. A short T_2^* can be as destructive as a short T_1 for a beautiful theory of non-linear effects in biology.

The determination of the T_1 and T_2^*'s of a state starts with the static spectroscopy of the state. If a particular transition is not heterogeneously broadened, the static lorentzian linewidth Γ_o of the transition (in energy units of wavenumbers, cm^{-1}) is given by [1]:

$$\Gamma_o = \frac{1}{c}\left[\frac{1}{\pi T_2^*} + \frac{1}{2\pi T_1}\right] \quad (1)$$

The classic problems in proteins is of course is that by static spectroscopy it is not possible to disentangle T_1 from T_2^* by static spectroscopy alone, and further that the vibrational lines in the IR spectra of a protein are inhomogenously broadened due to complex energy landscape of a protein [2]. It is possible that within this distribution of lines that there exist a set of states that are both spectrally narrow and have long dephasing times. In order to discover these states it is necessary to probe their dynamics by some form of site-selective spectroscopy.

STATIC SPECTROSCOPY

In fact, there is evidence from static spectroscopy at least that there exists a distribution of relatively narrow states within the broad envelope of amide I modes in proteins. We take as our example Mb, but other proteins show similar evidence (data not shown). Fig. 1 shows the spectrum IR spectra of myoglobin in a D_2O-d-glycerol buffer as a function of temperature. If we concentrate on the amide-I band, it is clear that the short wavelength side of the amide I band shows little temperature dependence, while the long wavelength (red) side shows the appearance of a new band as temperature is decreased, indicative of increasing hydrogen bond strength with decreasing temperature [3]. The amide I mode is due to the C=O stretch of the amide group, and is moderated by a weak hydrogen bond with neighboring hydrogen atoms of the primary amine groups, and with hydrogens of water if they are accessible to the amino acids. Those amino acid groups which are are buried within the protein and not in contact with the surface or the solvent would be expected to not have this temperature-dependent change in the amide-I frequency, thus we identify the blue-side part of the amide-I band with the internal amino acids, and the red-side amide-I band with the external, solvent exposed amino acids.

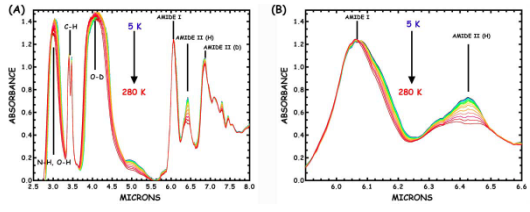

Figure 1: (A). The infrared absorption spectrum of sperm whale myoglobin as a function of temperature from 5 K to 300 K, from 2.8 to 8.0 microns. (B) The spectrum from 5.9 to 6.6 microns

We can make a further observation: the Debye-Waller factor S, a measure of the variance of the positions of atoms in a lattice [4], is also a function of the position of the amino acid in a protein. S is defined by: $S =< x^2 >$, where

$< x^2 >$ is the variance of the position x of atoms in the protein crystal. As a general rule, the interior parts of the protein have small S while those at the surface of the protein have large S. Thus, not only do the internal amino acids have small Debye-Waller factors but they also show less interactions with the solvent. One might then imagine that these internal amino acids have unusual dynamical properties compared to the amino acids at the protein surface which are held less tightly in place than the interior.

PUMP-PROBE MEASUREMENTS

At FELIX it turns out that the pulse width can be made as long as 10 picoseconds, giving rise to a linewidth of about 2 cm^{-1}, while still retaining about 1 microjoule of energy delivered to a 100 micron spot size. Further, at FELIX it is possible to deliver this pulse as a single pulse, since as we have painfully shown delivering too much energy via a series of micropulses at a rate higher than the diffusional cooling rate only leads to damaging the protein and introduction of complications [6]. Fig. 2 shows the heterodyne mixing signal of a long pulse from FELIX. This pulse has a duration of 10 ps and hence a spectral linewidth of 0.01 microns or 2 cm^{-1}. When such a long pulse is scanned across the amide I+II spectral region of Mb, from 5.8 to 6.5 microns, a remarkable thing happens. While ordinarily when pulses of duration 1 ps or shorter are used the typical transmission change observed for 1 uJ pulses focussed to 100 micron spot sizes is about 1% or less, if the spectral width is narrowed the signal enormously increases, by a factor of 50! Fig. 3 shows an example of the signals observed as a narrow pulse is scanned across the amide I+II region. Note that the huge enhancement is seen only for the amide I band, and only on the blue side of the amide I band.

This blue-side signal enhancement could be due to excitation of chromophore bands which lie within the amide I band of the protein, but this is probably not the case. Fig.4 shows a scan of the amide I region of lysozyme, an enzyme which has no chromophores. Not only does this scan show that a similar anomalous enhancement of the pump-probe signal occur on the blue side of the amide I band of this protein, it also shows that the shape of this enhanced signal depends on the protein. Of course, narrowing the spectral width has its costs in that the time resolution decreases. Fig. 5 shows what happens as the pulse width is changed to the time-domain response of the sample. When the pulse width is long the response of the protein, in this case myoglobin, looks like the autocorrelation of the pulse with no obvious lifetime. As the pulse is shortened, exciting more modes and putting less energy into each mode, the shape of the response qualitively changes with a clear lifetime effect coming in at longer times but less amplitude to the pulse. If the pulses are made as long as possible and the signal is observed at the spectral peak of the response, then varying the energy of the pulse by use energy attenuators shows that the transmission change is proportional to the energy of the

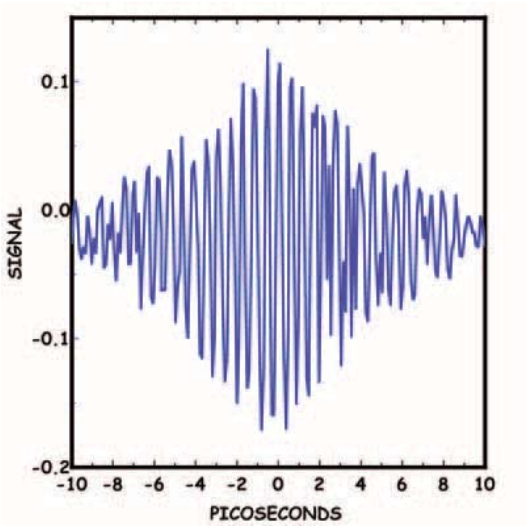

Figure 2: Heterodyne mixing of the pump and probe pulse used for these experiments.

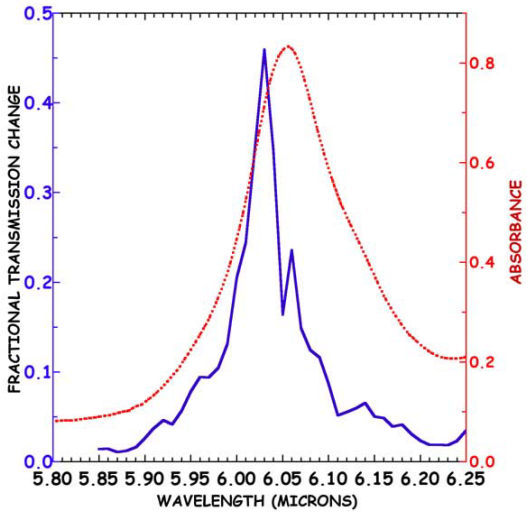

Figure 3: Wavelength scan of the transmission change vs. wavelength of the pump-probe signal seen for Mb in a 75% d-glycerol/25% D_2O solvent at room temperature (solid line). We have superposed the measured absorbance of this sample (dashed line).

pulse. Finally, the polarization of the pump pulse can be rotated with respect to the polarization of the probe pulse, these measurements shown in Fig. 6 reveal that the pump polarization must be parallel to the probe polarization for maximum signal.

It is difficult in this brief communication to reveal succinctly all of the data we have required, even more difficult to explain what is happening. We have already discussed how the heterogeneous broadening of the amide I

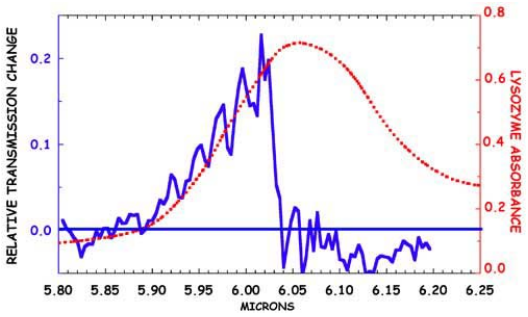

Figure 4: Scan of the pump-probe signal observed for the enzyme lysozyme (solid line). The absorbance of the sample is given by the dashed line.

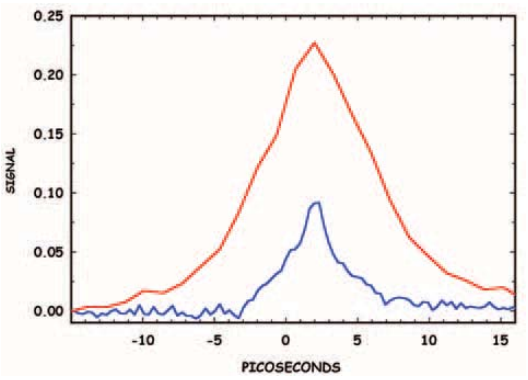

Figure 5: A time scan of the Mb signal at 6.04 microns as the pump pulse is scanned via the delay line over the probe pulse. The two traces are for a 10 ps and a 2 ps wide pulse.

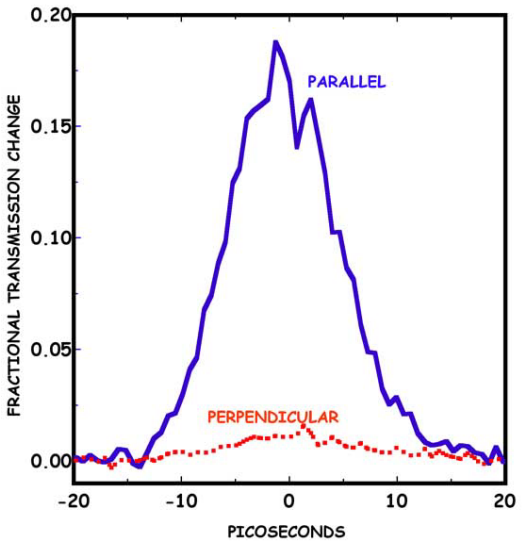

Figure 6: A time scan of the Mb signal at 6.04 microns as a function of the relative polarization between the pump and probe.

band can be roughly split into a blue side distribution of buried amino acids not in contact with the solvent and a red side distribution of solvent exposed amino acids. We also speculated that perhaps the blue side, buried amino acids with their small Debye-Waller factors might have long coherence times T_2^*. How can we begin to draw a quantitative connection between coherence dephasing times and the amplitude of these strange signals? The simplest model, which does require phase coherence of the excited state, is the phenomena called Rabi flopping [1]. In Rabi flopping a 2 level system is driven by a resonant electromagnetic wave with electric field strength E. We denote the strength of the electric dipole transition moment to be μ. Somewhat counter-intuitively, when such a two level system is driven coherently the population of the ground state $\rho_1(t)$ and the excited state $\rho_2(t)$ oscillates as:

$$P_1(t) = \frac{[\cos(\chi t) + 1]}{2} \qquad (2)$$

where $\chi = \frac{\mu E}{\hbar}$ is the Rabi flopping frequency. The surprising aspect of Rabi Flopping is of course the fact that a population can completely invert with time if driven resonantly and if the dephasing time T_2^* is much longer than χ. It is possible to characterize a pulse of such resonant light by an angle $\theta = \int \chi dt$ which represents for example the population change it creates. For example, a $\pi/2$ pulse would the equalize the ground and excited state populations. Such a pulse would of course result in a huge bleaching signal because there would be no net absorbance changes.

It is possible to estimate θ and hence the extent of transmission change possible. The FEL pump pulse had an energy of 1 microjoule, a pulse duration of 5 ps, and a focused diameter of 100 microns. This yields an optical electric field E of about 10^8 V/m. The amide I band is a strongly allowed transition, we assume the electric dipole moment is approximately 1e-Å. We compute then that a π pulse should take about 1 ps, IF the coherence time T_2^* is on the order of the longitudinal relaxation time T_1. Of course, dont know dipole moment, so this is just a guess but it does indicate that if a narrow state with long T_2^* exists, its population can be inverted. We can guess how the intensity of the observed bleaching signal signal will vary with θ. Since the potential transmission change in our case is about 1000% (a ten-fold increase in transmission since the sample has an optical density of about 1 at the maximum wavelength). Since we observe at most 50% changes the actual value of θ must be much less than 1 radian. Thus, we can expand the $\cos(\theta)$ term in Eq. 2 yielding:

$$P_1(\tau) \sim 1 - \frac{[(\mu E \tau/\hbar)^2}{4}] \sim 1 - \mathcal{E}_{pump}\frac{[\mu/\hbar]^2}{4} \qquad (3)$$

where we measure the response at the midpoint of pump-pulse, τ and $\mathcal{E}_{pump}$ is the energy of the pump pulse. The prediction then is that the transmission change which is proportional to $P_1 - 1$ would be proportional to the energy of the pump $\mathcal{E}$. In Fig. 7 we show that in fact the signal

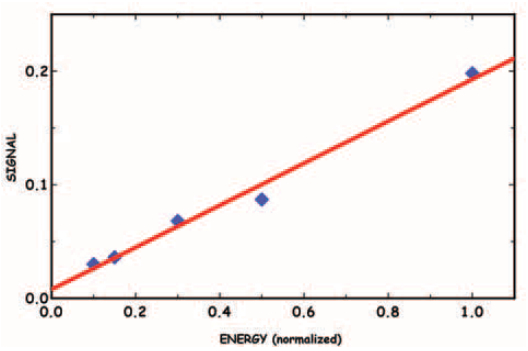

Figure 7: The dependence of the peak in the transmission signal with pump pulse energy for Mb.

is linearly proportional to the pump energy as predicted by Eq. 3.

We come back to the issue of Original Sin. We suggest that the T_2^*, the dephasing times, of the deeply buried amino acid band is very long, at least 20 ps. IIf so, then this part of the protein is an inherently quantum mechanical, strongly coupled anharmonic system. system. Perhaps we are addressing one aspect of the Original Sin problem in protein dynamics.

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