RF Absorption Involving Biological Macromolecules

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The fundamental intramolecular frequency of a globular protein can be obtained from the measurements of acoustic velocities of bulk protein matter. This lowest frequency for common size molecules is shown to be above several hundred GHz. All modes below this frequency would then be intermolecular modes or bulk modes of the molecule and surrounding matter or tissue. The lowest frequency modes of an extended DNA double helix are also shown to be bulk modes because of interaction with water. Only DNA modes, whose frequency is well above 4 GHz, can be intrahelical modes, that is, confined to the helix rather than in the helix plus surroundings. Near 4 GHz, they are heavily damped and, therefore, not able to resonantly absorb. Modes that absorb radio frequency (RF) below this frequency are bulk modes of the supporting matter. Bulk modes rapidly thermalize all absorbed energy. The implication of these findings for the possibility of athermal RF effects is considered. The applicability of these findings for other biological molecules is discussed.


Key words: molecular RF absorption; biological effects; water damping; energy transfer; athermal effects

INTRODUCTION

There has been much attention paid to theoretical mechanisms by which radio frequency (RF) irradiation may affect biological processes. One area that has not been extensively studied is whether RF can subtly effect the action of individual enzymes. In many biological molecules, physical displacement of atoms from one conformation to another is intimately connected to their biological function. For example, the conformation of hemoglobin is altered after one heme group absorbs oxygen in such a way that the three remaining heme groups become much more likely to absorb oxygen. This greatly increases the efficiency of hemoglobin as an oxygen transporter. The change in oxygen affinity is driven by the conformation change of the molecule which involves very localized changes in the conformation of the heme group, as well as change over larger sections of the molecule [Austin et al., 1975]. The conformational dynamics of the molecule can directly interact with its enzymatic function.

Conformation change involves atom displacements, as do vibrational excitations, and specific vibrational modes can be important in driving conformational change [Eyster and Prohofsky, 1977a,b; Moore et al., 1983; Lindsay et al., 1988]. If RF absorption altered the vibrational dynamics affecting conformation change, it could conceivably affect enzyme function. It is known that high temperature affects function, and higher temperature can be thought of as an increase in the excitation level of all vibrational modes, consistent with maintaining a thermal distribution. RF absorption could conceivably raise the excitation level of a few specific modes and affect function if they were the operative or biologically active modes.

In this article, we will explore the role RF irradiation may play in altering vibrational mode amplitudes, possibly leading to changes in conformation or other biological effects, in two biological molecules, myoglobin (MGb), and the DNA double helix. In both cases, we will generalize the findings to describe the effects in other systems. We will develop the analysis using vibrational dynamic information of these systems as (1) the vibrational modes are a complete basis set for analyzing any change in conformation, (2) there are theories for how mode amplitude can alter conformation, (3) vibrational modes provide a well understood mechanism for any

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possible coupling to RF fields, and (4) there is a well understood framework for analyzing how any possible energy absorption may be transferred between vibrational modes so that even if absorption occurs at some non biologically active mode it might still affect function.

The entire spectrum of vibrational modes in biological structures ranges from very low frequency modes that involve in phase motion over large segments, to very high frequency modes, which involve opposing displacements between adjacent atoms. The behavior of the lowest frequencies can be approximately calculated in the continuum approximation. That approximation is appropriate when the domain of “in phase motion” is so large that the atomic nature of matter is not important. One can then treat the problem in terms of compressions or torsions of bulk continuous matter. These continuum modes are a very small subset of the entire vibrational spectrum and are not sufficient to describe the localized displacements such as those of importance in the heme group in MGb.

A number of calculations have been carried out in the continuum approximation, for example, by Adair [2002]. He has shown that the absorption cross section, in the systems he explored, is small. He has also found that the relaxation of any absorbed energy would be fast ruling out resonant absorption. The continuum approximation, however, has to be used with care on the molecular level. We will show that it is appropriate for modes well up to tens of GHz. We will also show that all but the largest possible molecules are incapable of absorbing at RF frequencies into intramolecular modes. The net result is that any absorption could then be calculated using the approximations made by Adair. We will also discuss how any energy absorbed by continuum modes will be thermalized at a faster rate than it could be transferred to biologically active intrahelical modes. This leads to the conclusion that biological effects in molecules are limited to those determined by simply considering thermal effects of RF irradiation.

The first case studied will be the MGb protein, since this is the only protein system for which a nuclear resonant vibrational spectrum (NRVS) is available. These data can be used to make an unambiguous determination of the frequency, where the continuum compressional behavior ends and intramolecular behavior begins. The second case explored will be extended double helical DNA. DNA has been chosen because of the unique availability of Brouillon scattering data, which allows determination of the relaxation time for coupling of waves on the helix to the surrounding water of hydration. Those data allow accurate determination of the damping due to water.

MGb is an example of a compact globular protein. It has an internal stiffness believed to be similar to that of other globular proteins. We will find that, because of its stiffness, it has vibrational modes that can be thought of as having relatively high Q, but also because of that stiffness, its fundamental vibrational mode (the lowest frequency resonant mode) is relatively high. In a detailed calculation, its fundamental is calculated to be at a frequency of 187 GHz [Adair, 2002; B.K. Rai and E.W. Prohofsky, private communication, to be submitted].

DNA is long when stretched out and is, in principle, capable of supporting very low frequency vibrational modes. Because it is extendible, it is also in intimate contact with water in all of its native configurations. The damping of modes in DNA by water is investigated and the damping rules out resonant intramolecular behavior below 300 GHz. In the DNA and the MGb cases, modes exist below the frequencies of the molecular resonant modes, but they could only be modes of the bulk matter the molecules are embedded in, that is, intermolecular modes, not intramolecular ones.

The distinction between intramolecular modes and intermolecular or bulk modes of the background matter is important. The intramolecular modes are those with motion of atoms localized to the macromolecule itself. The bulk modes would be those that are long wavelength sound waves. Ultrasonic propagation clearly demonstrates that these low frequency sound waves exist in tissue. The possibility of an athermal RF absorption effect is greatly affected by the distinctions between bulk mode absorption and intramolecular absorption. In the former case, the absorbed energy is from the very beginning in modes that are strongly coupled to the large density of states making up the specific heat of the bulk matter. Thermalization of the absorbed energy is then very rapid. In the latter case, the energy is in the molecule that has biological function.

**ESTABLISHING THE LIMITS FOR CONTINUUM APPROXIMATION VALIDITY IN PROTEIN**

The absorption of electromagnetic energy by matter always depends upon mechanical motion of charged matter, such as the orbital motions of electrons in atoms or molecules, which must be resonant with X-rays or light, or the vibrational and spinning motions of relatively massive chemical groups that can absorb energy in the infrared and RF regions. Most molecular spectroscopy is conducted in the range above 10–100 cm⁻¹ (300 GHz–3 THz) because below these frequencies one usually sees only a continuum in absorption
rather than line spectra characteristic of isolated resonant vibrational motion.

All studies on large systems show that low frequency excitations are acoustic modes [Maradudin et al., 1963]. There are, however, special cases such as ammonia (NH₃) where the potential energy among the four charged atoms permits a slow tunneling between ammonia configurations. This slow process leads to additional resonances at the frequency of ~24 GHz at room temperature. The kind of motion displayed in this small molecule is not found in larger molecules as the large number of atom–atom interactions in the larger system will split the near degeneracy in energy needed for the two tunneling states. Conformation changes of a more complex nature, which are related to the ammonia mode, are still possible in large molecules but require a quite different analysis. Those that have been explored, in terms of what frequency would be involved in the dynamics of the change, all have initial frequencies in the sub THz region [Eyster and Prohofsky, 1977a,b; Prasad and Prohofsky, 1985; Lindsay et al., 1988].

Acoustic modes are observed to propagate in all kinds of matter, solids, liquids, gases, plasmas, etc. The reason is that one can always define some kind of modulus describing the reaction to some strain; and acoustic waves are solutions of the stress strain relations as shown in the Appendix. In crystalline solids, vibrational modes are found which are characterized as acoustic and optical [Maradudin et al., 1963]. The acoustic branches have linear dispersion, that is, a region characterized by an acoustic velocity. The lowest frequencies have the longest wavelength as the velocity \( v \) is the wavelength \( \lambda \) divided by the period \( T \), the frequency is \( v/\lambda \). The equation for the displacement of atoms as a function of position \( r \) in an acoustic wave is

\[
x = X \cos \left( \frac{2\pi}{T} t + \frac{2\pi}{\lambda} r \right) = X \cos(\omega t + qr)
\]

(1)

where \( X \) is the amplitude and \( q \) is the wave vector \( 2\pi/\lambda \). The \(-\) sign indicates wave traveling in the \(+r\) direction and the \(+\) sign, a wave traveling in the \(-r\) direction.

These traveling waves can also be expressed as standing waves using a simple trigonometric identity for \( \cos(\alpha + \beta) + \cos(\alpha - \beta) \). The sum of two waves of the same frequency traveling in opposite directions with the same amplitude is

\[
x = 2X \cos \omega t \cos qr
\]

(2)

where the wave oscillates in time, due to the \( \cos \omega t \) term, inside an amplitude envelope described by the \( \cos qr \) term. All thermodynamic and time averaged behavior is the same in either representation of the waves. Traveling waves are more useful for transport analysis and standing waves are more useful for fitting particular boundary conditions.

College physics textbooks [Cutnell and Johnson, 2001; Walker, 2002; Halliday et al., 2003] describe fitting linear waves to specific boundary conditions to make standing waves on a string of finite length, and for fitting acoustic waves in organ pipes. Open end organ pipes are fitted to finite length boundary conditions assuming antinode behavior at both open ends. The fundamental mode (lowest resonant frequency) is the one with one half of the wavelength equal to the length of the pipe. Similar fitting is done in insulating solids to determine the number of modes in calculating the specific heat in the Debye Approximation [Maradudin et al., 1963]. The Stefan–Boltzmann Equation [Landau and Lifshitz, 1958] for black body radiation uses a similar fitting scheme in its derivation, this time for light waves in a finite volume black body. The transformation between traveling and standing waves works for any linear harmonic system. The method is applicable to fitting acoustic waves in a macromolecule that is embedded in a larger continuum. The lowest frequency intramolecular modes in a molecule such as MGb embedded in bulk myoglobin will be closely approximated by fitting standing waves with antinode boundary conditions to the length of the molecule. The approximate fundamental would be that with \( \lambda/2 \) fitted to the diameter of the molecule.

Consider a molecule of diameter \( D \) embedded in bulk matter experiencing a long \( \lambda \) wave, where \( D < \lambda \). From Equation (1), it can be seen that the variation in \( x \) over \( D \) would be very small and in the limit \( D \ll \lambda \), all atoms in \( D \) would have the same displacement \( x \). The mean square displacement \( \langle x^2 \rangle \) would also be the same over distances \( D \), that is, over all the atoms of the single molecule. For harmonic waves, \( \langle v^2 \rangle = \omega^2 \langle x^2 \rangle \) where \( \langle v^2 \rangle \) is the time average of the velocity of the atoms in \( D \). Both the motion and displacement over \( D \) is the same for all atoms for a mode of this type. The molecule can be thought of as riding the wave. Sound waves are also found at long wavelength in lattice dynamics, where the analysis starts from atoms with specific interactions in specific conformations. When one matches microscopic and bulk parameters to the same material, the velocity of sound is the same in the two cases.

The assumption that modes at length \( \lambda \) have linear dispersion (constant acoustic velocity) and can be fit into matter as discussed above is the Debye Approximation [Maradudin et al., 1963] in lattice dynamics. It is found to hold as long as \( \lambda \) is long compared to interatomic distances. Another marker for Debye behavior is a quadratic frequency dependence of the density of states, which follows directly from linear dispersion.
The density of states for acoustic modes in the Debye Approximation \( D(\omega) \) is related to the mean displacement of any atom in the material is

\[
\left\langle x^2 \right\rangle = \frac{\hbar D(\omega)}{m \omega} \left[ \frac{1}{\exp(\varepsilon/\kappa T) + 1} + \frac{1}{2} \right]
\]  
(3)

where the term in the bracket is the Bose factor, a function of energy \( h\varepsilon \) over \( kT \), where \( h \) is Planck’s constant, \( f \) the frequency, \( k \) is Boltzmann’s constant, and \( T \) is absolute temperature. \( D(\omega) \) can also be written for linear dispersion as

\[
D_{\text{Debye}} = \frac{m_{Fe}}{2\pi^2 \rho v^3} \omega^2. 
\]  
(4)

The \( \omega^2 \) dependence is directly related to a constant acoustic velocity and the uniform density of states in \( q \) space by fitting to boundary conditions as described above.

\[
\nu = \left[ \frac{3v_l^3 v_t^3}{2v_l^3 + v_t^3} \right]^{1/3}
\]  
(5)

is the average velocity due to the fact that all polarizations of acoustic waves add to the density of states. \( v_t \) is the transverse polarized acoustic velocity and \( v_l \) is the longitudinal velocity [Maradudin et al., 1963].

**MGb**

There are two experimental observations from which unambiguous information about the motion of particular atoms in MGb can be found. One is incoherent neutron scattering (INS) which can be used to accurately determine the motion of hydrogen atoms [Settles and Doster, 1997]. The second is nuclear resonance vibrational scattering (NRVS) also called phonon assisted Mossbauer Effect [Sage et al., 2001; Achterhold et al., 2002]. Raman scattering and infrared absorption cannot give reliable information on specific atom motion as interpreting scattering or absorption amplitudes requires detailed solutions for RF electron coupling, electron phonon coupling, and correct mode assignments. Determining these interactions would only be possible with complete knowledge of the electron states and such knowledge is not as yet available.

NRVS gives accurate information on the motion of an atom with a Mossbauer resonance, in MGb that atom is the Fe atom when the Massbauer nucleus is \(^{57}\text{Fe}\). Samples with high concentrations of \(^{57}\text{Fe}\) have been analyzed by NRVS. As shown by Achterhold et al. [2002] in a sample of bulk MGb, the motion and displacements of the H atoms from INS are identical to that of the Fe atom from NRVS for lowest measured frequencies up to 360 GHz at low temperature and up to 240 GHz at room temperature. Since the H atoms are at a large number of positions in the molecule, many far from the Fe atom, the entire molecule must be moving coherently for such different mass atoms to have identical motion. Coherent motion over the molecule is the signature of acoustic motion for a molecule in an acoustic wave with wavelength large or at least comparable to the diameter of the molecule. The vibrational modes below 240 GHz are then simple acoustic modes in bulk MGb. As we will see at the highest frequencies in this range, the wavelength is comparable to and even less than the single molecule size.

The second observation from both INS [Settles and Doster, 1997] and NRVS [Achterhold et al., 2002] is that the density of states as a function of frequency is quadratic over the same range of 0–240 or 360 GHz, again depending on temperature. This also is only compatible with a dispersion relation in that region that is linear in wave vector, that is, acoustic modes.

The density of states can be used to determine the mean acoustic velocity from Equation (4). Achterhold et al. [2002] has found a velocity of 1605 m/s at 300 K in MGb. This can be compared to other measurements of the longitudinal velocity of ultrasound generated by laser pulses [Edwards et al., 1990] of 1828 m/s in MGb. This can be compared to other measurements of the longitudinal velocity of ultrasound generated by laser pulses [Edwards et al., 1990] of 1828 m/s in hemoglobin. Using the mean velocity of 1605 m/s and the value for the longitudinal velocity from laser pulse measurements, one can estimate the transverse velocity using Equation (5). The value of the velocities of the two transverse branches, assuming they are equal, is 1526 m/s.

**VIBRATIONAL MODES IN A FINITE MOLECULE**

The frequency versus wavelength relation of Equation (5) is appropriate for a molecule embedded in similar bulk matter or for one that is very long compared to its other dimensions with \( \lambda \) along the long direction. It is inappropriate for a roughly spherical molecule in vacuum or embedded in material with elastic properties that are quite different from it. The frequency for a spherical molecule in vacuum was displayed in a simple form by Ford [2003] and it is

\[
v = 48 \text{ GHz} \left( \frac{10 \text{ nm}}{D} \right) \left( \frac{c_p}{1500 \text{ m/s}} \right) f \left( \frac{c_p}{c_s} \right)
\]  
(6)

where \( D \) is the diameter in nanometers, \( c_p \) is the compressional acoustic velocity that characterizes the bulk properties of the molecule, and \( c_s \) is the
corresponding shear velocity. The solution requires spherical Bessel functions and has a numerical factor \( \frac{f}{c} \), which is a function of the ratio of the compressional to shear velocities and depends on the index of the mode. For the lowest frequency mode, which has quadrupolar symmetry, the factor \( f \), for the ratio of \( c' \)'s found for MGb is greater than 5. The study also reports a spherically symmetric mode which is not a true solution [Lamb, 1988; Savriot et al., 1996]. As almost all compact biological molecules are less than 10 nm, the first fraction is less than one. The compressional velocity of the molecule is larger than water (1500 m/s) and the second fraction is larger than one. For \( f = 5 \), the calculated frequency is

\[
\nu = 288 \text{ GHz} \left( \frac{10 \text{ nm}}{D} \right).
\]  

(7)

Using 4 nm as the diameter of MGb, the frequency is 720 GHz.

The spherical molecule has a higher frequency fundamental than would occur for a molecule of similar dimension but not spherical in shape. The lowest frequency would occur for a molecule that is very long compared to its cross section. For such a molecule, the best fit would be using Equation (2) and is similar to fitting a sound wave in an organ pipe. In this case, the wave could be either a compressional wave or a shear wave. The shear wave with the slower velocity would give the lowest possible frequency. In that case, the frequency would be

\[
\nu = \left( \frac{728}{D} \right) \text{ GHz}
\]  

(8)

where \( D \) is \( \lambda/2 \) in nm. For a 4 nm molecule, this would be 182 GHz. MGb is roughly \( 4 \times 4 \times 2 \) nm in size. Any molecule would have a fundamental between these two extreme cases of Equations (7) and (8). This minimum frequency mode would be the fundamental mode for absorbing RF if a dipole moment is associated with the mode.

We have assumed that the compressibility and shear are the same inside the molecule as in the bulk accumulation of many molecules. Although a given protein molecule is chemically connected by strong valence bonds, those bonds alone do not determine the three dimensional conformation or overall stiffness. The linear protein chain still has much flexibility to fold in different ways. The three dimensional conformation is stabilized by “nonbonded” forces such as electrostatic, van der Waal, and water binding effects. The forces that hold large numbers of molecules together as bulk matter are these same nonbonded interactions. The result is that the moduli determining acoustic behavior is likely the same in a single molecule as in bulk matter of many molecules; this is indicated to be the case by the smooth Debye behavior from frequencies requiring wavelengths of the size of the single molecule to wavelengths much larger than that of the single molecule.

One would be also interested in how inclusions of regions of water in proteins would affect the frequency of the fundamental mode. The velocity of sound in seawater is 1522 m/s. This is a longitudinal velocity as water does not support shear waves. It is slower but comparable to the 1828 m/s longitudinal velocity and very close to the 1526 m/s transverse velocity of bulk MGb. The combined acoustic velocity of waves in a mix of water and protein would lie in between the two separate component velocities. The combined velocities should be no less than the 1526 m/s velocity assumed for transverse waves. Thus inclusion of regions of water inside a protein will not lead to fundamental frequencies lower than that given by Equation (8).

**DOUBLE HELICAL DNA**

In principle, a macromolecule that is very long, such as a long strand of double helical DNA, can support vibrational modes with long wavelengths and therefore low frequencies. There are, in principle, modes in long double helical DNA with frequencies from several thousand cm\(^{-1}\) all the way down zero. The higher frequency modes involve relative motion between nearby valence bonded atoms. These stretch the strong valence bonds, leading to high frequency oscillations. The lowest frequency modes are acoustic modes as they involve motion in which whole base pairs move as a unit with very little relative motion between neighbor atoms [Mei et al., 1981; Prabhu et al., 1988].

The modes are categorized as longitudinal, torsional, and transverse. Acoustic modes extend up to about 900 GHz in DNA but optical modes cross over the acoustic branch at 8 cm\(^{-1}\) or 240 GHz [Prabhu et al., 1988]. Any absorption by the helix of RF energy below 240 GHz would have to occur by absorption to acoustic modes. At physiological pH, the helix has a net charge of \( -2e \) per base pair. This charge is neutralized by a \( +2e \) charge on attracted counter ions. Because the counter ions are not strongly bonded to the helix, they do not cancel the negative charge under all dynamic conditions. This can lead to a dynamic dipole moment oscillating at the frequency of the acoustic mode. The dipole moment can, in principle, interact with an RF field to absorb energy.

If the helix were stretched out in a line, there would be no net absorption due to the difference
between the DNA acoustic wavelengths and the RF wavelengths. Couplings between mismatched wavelengths have regions absorbing energy from the E field and other regions pumping energy into it, the net cancels. Net absorption could only occur if the linear symmetry of the helix were broken, for example, a segment between bends which had length just equal to 1/2 the helical acoustic mode wavelength. Then the cancellation in absorption due to wavelength mismatch would not occur. Since DNA is known to have secondary and tertiary structures with segments in various sizes, absorption may be possible. Kohli et al. [1981] calculated the absorption of such a segment, in vacuum. Since DNA requires counter ions and water of hydration to remain in its double helical conformation, that calculation is unrealistic.

A calculation of absorption, which included water and salt effects, was carried out by Van Zandt [1986]. His study did show somewhat narrow absorption lines in the presence of water at microwave frequencies and has been referred to many times to indicate that resonant absorption by DNA has been predicted. The calculation, however, was carried out with the unrealistic assumption of a helix–water relaxation time of 300 ps. The appropriate relaxation time had already been measured in pioneering work by Tao et al. [1988] through measurements of line widths of modes in oriented double helical DNA films by Brillouin scattering, using a highly accurate 9 pass tandem interferometer. The relaxation time was measured to be 39 ps for helix to the first hydration layer and a few ps for the coupling between the first layer and surrounding layers of water. If one uses the equations of the Van Zandt study with a proper relaxation time of 39 ps, no resonant absorption appears in the microwave region.

**INTERACTION OF THE HELIX WITH WATER**

Vibrational modes below 240 GHz, predicted to exist in DNA [Kohli et al., 1981; Van Zandt, 1986], are acoustic modes that can be expressed as propagating or standing waves on the helix. The extended helix has much surface in contact with water and attracts layers of water as bound water or water of hydration, which is present even in dry samples that have little or no free water. As shown in the Appendix, the loss mechanism in DNA is qualitatively different at high frequency (\(\omega \tau \gg 1\)) than it is at lower frequencies. The important parameter is the product of angular frequency \(\omega\) and the relaxation time \(\tau\), which characterizes the speed at which the water can reach equilibrium accommodating to the existence of the wave in DNA.

The determination of the relaxation time is best done by examining the frequency dependent damping due to water around a DNA double helix as described by Equation (A16). The observed relaxation time for damping to the first hydration layer water was measured to be 39 ps by Tao et al. [1988]. Equation (A16) is a good approximation to the damping in the limit of weak coupling, and it can be seen to approach very close to the more exact high frequency Equation (A15) at high frequency. Equation (A16) also approaches very close to the more exact low frequency Equation (A14) at low frequency. Only the coefficient of the \(\omega \tau\) dependent factor differs somewhat in the two cases.

Using Equation (A16) for a fixed relaxation time, the frequency dependence of the damping for a coherent acoustic wave is displayed in Figure 1. The damping seems to drop at low frequencies, but this is not the damping for a pure helical mode. It does not mean that low frequency intrahelical acoustic modes are undamped. It is a helical–water mode that has reduced damping. From Equation (A4), we see that the distribution of excitations in the water at low frequencies does not differ from what it would be in perfect equilibrium with the presence of the wave at zero frequency. For water attached to the helix, this would mean that the water is following the motion of the helix atoms. The water is entrained in the wave and the wave becomes a coherent wave of both water and DNA. The drop in damping at low frequency is because the water is no longer damping the wave; it has become part of it. The high frequency wave velocity \(c_\infty\) is the velocity of a pure DNA wave not loaded with water. The low frequency velocity \(c_0\) is the velocity of a different wave, one of water and DNA oscillating coherently.

The effect of water coupling is then to eliminate the existence of a pure intrahelical DNA mode to
frequencies where \(\omega \tau \approx 1\). At these frequencies, the damping by water of the pure DNA mode is becoming large, as the water is not fully moving with the wave, and viscosity occurs. The damping is 1/2 its maximum at \(\omega \tau = 1\) which occurs at 4 GHz for DNA.

If the DNA and its first layer of water are embedded in more water, one would have to repeat the analysis in the Appendix for the mid to low frequencies with the combined DNA–first water layer as the coherent wave, interacting with water layers further out. That interaction has a relaxation time an order of magnitude less than 39 ps, and the combining of the wave to the bulk water would be even more rapid and more complete.

To construct a system like the one in which the Brillouin Scattering was observed [Tao et al., 1988], instead of a helix in bulk water, the helix and first layer would be in a relatively dry film. The first water layers of the many helices are, however, in close contact. The fast water–water relaxation would dynamically connect the helix water layers, which are also connected to the helices, leading to a coherent water of the whole accumulation of helices and water, that is, to a bulk matter wave in the film. The modes observed in the Brillouin and Raman Scattering experiments [Tao et al., 1986, 1987, 1988; Tao and Lindsay, 1988, 1989a,b] were at tens of GHz in frequency and not overdamped. That these modes were bulk modes, however, is apparent from the observation that longitudinal acoustic waves were observed to propagate in the film perpendicular to the orientation of the helices in a similar manner as propagation parallel to the helix orientation. An individual helix can only support acoustic intramolecular modes parallel to its orientation. In wetter samples, the coupling to larger amounts of water rules out seeing any resonant absorption at any frequency.

RF absorption in dry DNA will be to the bulk matter of the system, that is, to acoustic waves in the film, not to pure intrahelical modes. In a complex of many elements of tissue, the coupling would be to bulk waves of the entire tissue.

**GENERALIZATION OF FINDINGS**

We have discussed two specific molecules and found that absorption below several hundred GHz would not be resonantly absorbed into an intramolecular mode in either case. Any absorption that did occur would, from the outset, be into bulk modes of the material the molecule was embedded in. Bulk modes are strongly coupled to other bulk modes, and the thermalization of any absorbed energy would be primarily to the large specific heat of the bulk matter rather than to the smaller density of states and smaller specific heat of a single molecule.

The question arises as to how representative of all biological molecules these two cases are? The MGb results should be roughly applicable to all compact globular proteins. The moduli determining the acoustic velocity are averages over a large number of “non-bonded” interactions between residues of the MGb. These averages should be pretty much the same in other globular protein as the interactions which determine the moduli all involve the same amino acids on average. The specific amino acids do change but the variation is unlikely to alter the globular protein acoustic velocity by much. All reasonably sized compact globular proteins would have fundamental frequencies above hundreds of GHz. They would be incapable of resonant absorption into an intramolecular mode at lower frequency.

The results for DNA should also hold for other molecules that are as extended and surrounded by water. Modes at angular frequencies lower or comparable to the reciprocal of a relaxation time would not be intrahelical modes. There is no reason one would expect the relaxation time for nearby water to be much different from that found by Tao et al. [1988]. The interaction with water would mix water and other elements making up the bulk matter wave. The result would be that even if there were energy absorbed from an RF field it would be into bulk modes rather than modes within the molecule.

**LOW FREQUENCY MODES THAT ARE NOT SIMPLE BULK-LIKE MODES**

There are cases that obviously do not fit this analysis. There could be large complexes of compact globular protein that are connected by weak bonds in such a way that they can support low frequency modes, with some segments oscillating against other segments. One example would be the barrel shaped pore protein complexes. Another would be proteins connected by a weak hinge like region. The motions in question could be described in terms of continuum classical models of the oscillation of large segments such as those by Adair [2002]. The large segments would move in water and would be subject to classical viscosity loss and would lead to overdamped behavior, which would again lead to coupling any absorbed energy to bulk matter.

Another mode that does not fit the patterns described which we have found in MGb [B.K. Rai and E.W. Prohofsky, private communication, to be submitted] and also occurs in hemoglobin is a mode in which the heme group oscillate in the protein pocket.
that surrounds it. In these molecules, the heme group is only loosely coupled to the surrounding protein and can rotate at low frequency around the one valence bond that connects it to its surroundings. In our calculations, the frequency of this mode is at 184 GHZ and is the lowest mode in MGb. This mode has very little dipole moment associated with it and would not absorb appreciable EM radiation. Modes of this kind are unlikely to be lower than this frequency in other molecules.

ENERGY TRANSFER FROM BULK MODES TO BIOLOGICALLY ACTIVE MODES

The absorption of RF energy, in all cases discussed, is to modes of bulk matter rather than to intramolecular modes of a specific molecule. The question still remains as to whether that energy can be transmitted to other modes in a way that can athermally alter biological function. It is known that at high enough temperature, biological function is altered. The question then is, can energy be transferred more efficiently to active modes in a way different from assumptions related to thermal heating. The question can be answered because there is a considerable amount of knowledge about energy transfer between modes in bulk systems [Maradudin et al., 1963].

Since the intramolecular modes of the molecule have been shown to all be at higher frequencies than those of the bulk matter, the transfer to active modes must involve scattering to higher frequency. The leading scattering terms for lattice modes involve three phonons and the net rate of transfer involve the activation levels of the modes that are out of equilibrium. If there were resonant absorption, the absorbing mode would have the greatest level of activation. The leading upconversion process would then be frequency doubling, that is, that process in which the initial two phonons were of the RF absorbing mode leading to the third created phonon of twice the frequency. The largest transfers would then be to modes nearby in frequency. The high frequency active modes would then only have energy transferred to them when modes were populated out of equilibrium up to near the active mode frequency by pumping up all the modes at frequencies in between. This transfer of energy through a large number of modes is exactly the process of thermalization. For an athermal effect, there would have to be a transfer to the one mode greatly in excess of the transfer of energy to all other modes. This could only happen by a very unusual strong coupling between a bulk and an intramolecular active mode. Since they are so different in character, this is extremely unlikely.

SUMMARY

Individual protein molecules have a lower limit to the frequency intramolecular vibrational mode they can support resonantly. An estimate of this frequency, based on data from MGb, is given in Equation (7). No resonant RF absorption could occur to a single protein molecule of this size below this frequency. Bulk intramolecular modes are present at lower frequency and if any RF absorption occurred it would be to bulk modes.

A study of the damping of extended DNA shows that damping of intrahelical modes by water is very large for modes above 4 GHz. For lower frequencies, the coupling to water causes a mixing of water into the DNA acoustic mode. This coupling extends through the water to admix other elements of bulk matter. The resulting mode is an acoustic wave in the bulk matter rather than an intrahelical mode of the DNA.

The relaxation of any energy absorbed by bulk modes is rapidly thermalized to the total specific heat of the bulk matter.

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REFERENCES


APPENDIX

In this appendix, we develop a formalism for taking into account any number of entities that might interact with an acoustic wave propagating along a system like the double helix. The approach assumes a phase coherent acoustic mode, which interacts with excitations of the rest of the system. The approach allows treatment of both the damping to water and the transfer of energy to other modes including quantum excitations, that is, high frequency modes which satisfy \( \hbar f > KT \). The approach, second viscosity [Landau and Lifshitz, 1959; Prohofsky, 1967] is very useful in that we would not have to specify fine details of the modes involved in damping. The energy \( E_i \) of a background mode is altered by the compression or rarefaction, which the coherent acoustic wave imposes on the system. The \( E_i \) would then vary in time and space with the frequency and wavelength of the coherent mode. If the energy of the modes varies the equilibrium occupation numbers \( n_i^0 \) will also vary. A difference can occur between the instantaneous \( n_i \) and equilibrium \( n_i^0 \) excitation of the interacting modes. Relaxation processes are not instantaneous and their behavior in time can be characterized by relaxation times.

Let \( n_i \) be the instantaneous occupation number for mode \( i \). Let \( n_i^0 \) be the instantaneous equilibrated expectation value for \( n_i \) that takes into account the shift in its \( E_i \) due to the local compression or rarefaction of the acoustic mode. Assuming small variation in \( n_i \),

\[
\begin{equation}
 n_i^0 = n_i^0 + n_i^{01}e^{-i \omega t}. \tag{A1}
\end{equation}
\]

Similarly,

\[
 n_i^1 = n_i^{00} + n_i^{01}e^{-i \omega t}. \tag{A2}
\]

The relaxation time approximation requires that

\[
\frac{\partial n_i}{\partial t} = -\frac{n_i - n_i^0}{\tau_i} \tag{A3}
\]

where \( \tau_i \) is the time for relaxation of mode \( i \) from some nonequilibrated value to its equilibrium value. Then

\[
 n_i^1 = \frac{n_i^{01}}{1 - i\omega \tau_i}. \tag{A4}
\]

To use the most general form for the acoustic mode, that is, not specify longitudinal, transverse, etc., we will use generalized stress strain notation. Assume the acoustic mode has a varying stress \( T \) and strain \( S \). The stress will be affected by the distribution of excitations in the levels of all other modes. This is a reflection of the fact that a system can become softer at higher levels of excitation, that is, higher temperature. By straightforward chain law differentiation

\[
 \frac{\partial T}{\partial S} = \left( \frac{\partial T}{\partial S} \right)_{n_i} + \sum_i \left( \frac{\partial T}{\partial n_i} \right) \frac{\partial n_i}{\partial S}. \tag{A5}
\]
Using Equation (A4), we can set
\[ \frac{\partial n_i}{\partial S} = \frac{1}{1 - i\omega \tau_i} \frac{\partial n_i^0}{\partial S} \] (A6)
substituting into (A5)
\[ \frac{\partial T}{\partial S} = \left( \frac{\partial T}{\partial S} \right)_{n_i} + \sum_i \left( \frac{\partial T}{\partial n_i} \right) \frac{1}{1 - i\omega \tau_i} \frac{\partial n_i^0}{\partial S}. \] (A7)

From Equation (A7), one can see that one gets a different contribution to the sum from each relaxation mode depending on whether \( \omega \tau_i \) is large or small.

Consider a case where only one mode \( i \) is important in damping the coherent mode, that is, only one \( \frac{\partial T}{\partial n_i} \) or \( \frac{\partial n_i^0}{\partial S} \) is significant, leading to one significant relaxation time \( \tau \).

From Equations (A5) and (A6)
\[ \frac{\partial T}{\partial S} = \frac{1}{1 - i\omega \tau} \left[ \left( \frac{\partial T}{\partial S} \right)_{n_i} + \sum_i \left( \frac{\partial T}{\partial n_i} \right) \frac{\partial n_i^0}{\partial S} - i\omega \tau \left( \frac{\partial T}{\partial S} \right)_{n_i} \right] \] (A8)
Equation (A8) can be written as
\[ \frac{\partial T}{\partial S} = \frac{1}{1 - i\omega \tau} \left( \frac{\partial T}{\partial S} \right)_{eq} - i\omega \tau \left( \frac{\partial T}{\partial S} \right)_{n_i} \] (A9)
where
\[ \left( \frac{\partial T}{\partial S} \right)_{eq} = \left( \frac{\partial T}{\partial S} \right)_{n_i} + \sum_i \left( \frac{\partial T}{\partial n_i} \right) \frac{\partial n_i^0}{\partial S}. \] (A10)

First consider the case \( \omega \tau \gg 1 \) where the second term in Equation (A9) dominates and
\[ \frac{\partial T}{\partial S} \approx \left( \frac{\partial T}{\partial S} \right)_{n_i}. \] (A11)
which is just \( \frac{\partial T}{\partial S} \) when \( n_i \) is frozen, that is, no transitions to equilibrium occur during a period of the coherent wave. The compressions and rarefactions occur in a system which has the same frozen excitation distribution. Next consider the other limit when \( \omega \tau \to 0 \). In that case, \( \frac{\partial T}{\partial S} \) is just that given in Equation (A10). The transitions to equilibration for \( n_i \) are fast enough to keep up with the energy shifts and \( n_i = n_i^0 \) at each point in the cycle. The wave is propagating in a system that has excitations constantly in equilibrium appropriate to having a coherent wave present.

Since the velocity of an acoustic wave is given by \( c^2 = \frac{\partial T}{\partial S} \), we can rewrite Equation (A9) as
\[ c^2 = \frac{1}{1 - i\omega \tau} \left( c_0^2 - i\omega \tau c_\infty^2 \right) \] (A12)
where \( c_0^2 \) is the right hand side (rhs) of Equation (A10) and \( c_\infty^2 \) is the rhs of Equation (A11). The wave vector of the coherent wave is \( \omega/c \); in the limit of \( \omega \tau \ll 1 \), it is
\[ k_o \approx \frac{\omega}{c_0} + \frac{\omega \tau}{2c_0^3} (c_\infty^2 - c_0^2) \] (A13a)
and the damping \( \alpha \) is
\[ \alpha_0 \approx \frac{\omega \tau}{c_0^3} (c_\infty^2 - c_0^2). \] (A13b)
In the limit \( \omega \tau \gg 1 \), the wave-vector is
\[ k_\infty \approx \frac{\omega}{c_\infty} + i \frac{(c_\infty^2 - c_0^2)}{2c_\infty^3 \tau} \] (A14)
and the damping is
\[ \alpha_\infty \approx \frac{(c_\infty^2 - c_0^2)}{2c_\infty^3 \tau}. \] (A15)
In the limit of
\[ \frac{(c_\infty - c_0)}{c_0} \ll 1 \]
\[ \alpha_i \approx \frac{(c_\infty - c_0)}{c_0^2} \frac{\omega \tau \omega}{1 + \omega^2 \tau^2} = \frac{(c_\infty - c_0)}{c_0^2 \tau_i} \frac{\omega \tau \omega}{1 + \omega^2 \tau^2} \] (A16)
where \( \omega^2 \tau^2/(1 + \omega^2 \tau^2) \) contains all the frequency dependence of the damping of the coherent wave by the \( i \)th excitation. The dimensionless factor is displayed as a function of the product of \( \omega \tau \) in Figure 1. It displays the frequency dependence of acoustic wave damping for constant \( \tau \). The additional \( \tau \) dependence is the same as that in the \( \omega \tau \gg 1 \) limit of Equation (A15). The total damping for many background modes all weakly coupled would be approximately the sum of Equation (A16) over all modes \( i \).

To understand loss in a phase coherent semiclassical vibrational wave, consider first looking at a point in the cycle of the wave where the energy is all kinetic energy. The vibrational cycle then converts the kinetic energy to potential energy by compressing the system against a modulus that describes the stiffness of the system. At a still later time, 1/4 of the period later, the potential energy is reconverted to kinetic energy by expanding from the maximum compression. Loss would occur if there is less kinetic energy after completing a compression–rarefaction cycle, 1/2 the
period, than there was at the beginning. This is possible as the modulus is a complex function of the system excitations $n_i$ and may be softer on expansion then it was on compression. The inability to rebuild the kinetic energy causes loss each half cycle.

In the limit $\omega \tau \gg 1$, no changes in the $n_i$ occur, and the energy converted into potential energy will be the same as that converted back out of it over a cycle, as the modulus was the same over all parts of the cycle. In the other limit, $\omega \tau \ll 1$, the $n_i$ do change during the cycle; but as they are always in instantaneous equilibrium, they are again the same at each point going into the compression as at the same point on the way back out. The integrated transfer between kinetic and potential is still equal and no net loss occurs. Loss occurs when some $n_i$ change so that the modulus is different at the same point in the two quarter cycles and the integrated transfer back to kinetic energy is less when coming out of a compression than on the way in.

For $\omega \tau > 1$ some changes in $n_i$ occur and the modulus is not quite constant over the cycle. The more transitions that occur, the greater the change in modulus and the greater the loss. In this limit, the damping is proportional to $\tau^{-1}$, as shorter $\tau$ means more transitions. For $\omega \tau < 1$, the transition rate fails to keep up with equilibration, that is, $n_i$ departs from $n_i^0$, and the modulus changes are out of synchrony with the cycle. In this limit, it is the inability to keep up with equilibration, that is, the fact that the transitions are not quite fast enough that causes loss. The lack of fast enough transitions gives the $\tau$ dependence, since longer $\tau$ means fewer transitions. In the limit $\omega \tau \ll 1$, energy is transferred from the coherent wave to the $n_i$ modes during part of the cycle, but is transferred back during another. There is, therefore, no net damping, but the wave includes the $n_i^0$ changes, which become part of the coherent wave. Note that the velocity of the wave $c_0$ in the $\omega \tau \ll 1$ limit is different than that when the wave does not have the admixture of the coupled background mode. In the case of DNA coupled to water, the wave would be mass loaded by the coupled water and the modulus is also a combined one. The velocity of the water–DNA coupled wave would be expected to differ from a wave in DNA uncoupled to water.