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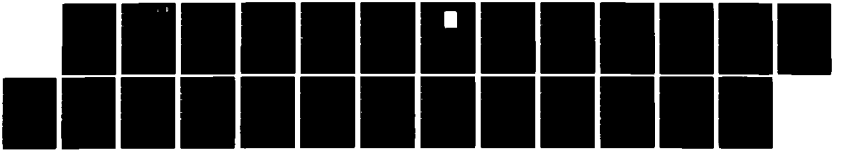

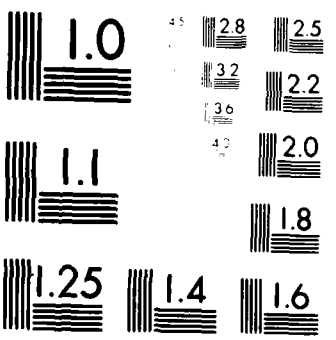

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**Abstract.**
We have used inelastic laser light scattering to probe the vibrational modes of DNA that lie in the microwave frequency region. We have studied acoustic (collective or sound-wave) vibrations in the region 7 - 20 GHz. in DNA films as they are hydrated. Taken together with an extensive study of the other physical properties of these films, these data show that strong interactions between phosphates dominate the dynamics in hydrated fibers. We have also studied the relaxation of the hydration shell using Brillouin scattering. At GHz. frequencies, water couples strongly to acoustic vibrations. The viscoelastic transition frequency of the water at 'primary' sites is about 4 GHz. while the secondary hydration shell undergoes this transition at about 80 GHz. Between these frequencies, there are coupled excitations of the DNA and its hydration shell which may account for the observed resonant microwave absorption. We have searched for direct spectroscopic evidence of these excitations without success. It is possible that an applied coherent microwave field must be applied to drive them.

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# Spectroscopic Search for Resonant Excitation of DNA by Microwaves.

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# Spectroscopic Search for Resonant Excitation of DNA by Microwaves.

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**Abstract.**

We have used inelastic laser light scattering to probe the vibrational modes of DNA that lie in the microwave frequency region. We have studied acoustic (collective or sound-wave) vibrations in the region 7 - 20 GHz. in DNA films as they are hydrated. Taken together with an extensive study of the other physical properties of these films, these data show that strong interactions between phosphates dominate the dynamics in hydrated fibers. We have also studied the relaxation of the hydration shell using Brillouin scattering. At GHz. frequencies, water couples strongly to acoustic vibrations. The viscoelastic transition frequency of the water at 'primary' sites is about 4 GHz. while the secondary hydration shell undergoes this transition at about 80 GHz. Between these frequencies, there are coupled excitations of the DNA and its hydration shell which may account for the observed resonant microwave absorption. We have searched for direct spectroscopic evidence of these excitations without success. It is possible that an applied coherent microwave field must be applied to drive them.

## 1. Introduction

The original goal of this work was to examine the inelastic light scattering spectrum of DNA fragments as they were illuminated by microwave radiation at one of the resonant absorption frequencies reported by Edwards et al.[1] as part of a collaborative effort with th Maryland group. That group has, as yet, not been able to make its contribution to the proposed collaboration. Nonetheless, we have learned a great deal about microwave excitations of the double helix from light scattering studies alone, and we will survey this work in this report. We still believe that a 'double resonance' experiment (i which light scattering is used to probe for microwave pumping) would be of value for reasons we will outline.

Our work on the gigahertz vibrations of DNA has covered the areas listed below - much of it is (or will be) published, and will therefore be covered only superficially here; more details of unpublished work will be given. An up to date survey is contained in

recent contribution to a conference proceedings included here as an appendix. The following projects are of relevance to the bioelectromagnetics program:

- A study of low lying collective modes in DNA fibers and films as they are hydrated.

- A study of the coupling of the hydration shell to DNA acoustic vibrations at GHz. frequencies.

- A search for acoustic resonances of the type reported by Edward et al.[1]. Using the same plasmid, we searched for inelastic light scattering signals from the acoustic modes believed to be responsible for the microwave absorption peaks. We also searched for a possible coupling between GHz. soundwaves and these modes.

We end this introduction by pointing out that over the past few years we have developed a unique array of spectroscopic instrumentation for studying vibrational modes between a few hundred GHz and a few hundred MHz. We believe this frequency interval to be central importance in biology, and welcome any collaborative proposal that would exploit the facilities we have here.

**2. Soft modes and acoustic modes - the influence of hydration.**

Three types of motion of DNA might be responsible for microwave absorption:

a) Resonant local modes which can lie as low as a few hundred MHz - We have reported possible observation of such a mode associated with the chain terminus[2], and new investigations of this mode are underway at present.

b) Acoustic modes on long chain polymers - the mode frequency depends on the chain length and the speed of sound, although there appears to be a lower frequency cut-off at a few GHz (see below). We have measured the speed of sound in DNA at a few water contents initially[3], but subsequently over a wide range of hydration [4,5,6] (and see the Appendix). The speed of sound (and thus any frequency associated with these modes) softens dramatically as water is added to fibers. This is a consequence of changes in interactions between adjacent double helices in the solid state (see below).

c) Optic modes (internal vibrations) which soften to drive a conformation change - At first, experiments appeared to indicate that the soft mode proposal[7] was essentially correct[8]. Low frequency Raman bands softened as the A to B transition occurred[8,9]. However subsequent work showed that these modes were dominated by the presence of strong inter double-helical bonds in the fibers and films. Indeed, so strong are the attractive interactions between DNA molecules in the solid state, that highly crystalline samples are almost impossible to

dissolve completely in water, residual crystallites causing the remarkable anisotropic swelling of these materials[10].

The nature of the interhelical bonding responsible for some of this behavior is discussed in the Appendix. However the following points should be noted:

- the interhelical contacts appear to form as a result of charge clusters (at high electrolyte concentration) around the highly negative O-2 atom of the phosphate group. In the solid state, these contacts are strong enough to drive the B to A transition. In the appropriate polymer at high salt concentration but otherwise low polymer concentration, such contacts between phosphates **on the same helix** will drive the B to Z transition.

- the mechanical (and hence microwave absorption) properties of the polymer are altered radically by such contacts. We have little experience of work with nucleosomes, but some experiments indicate that nucleosomal DNA is more like relatively dry fiber DNA[11]. Thus we expect very different interaction mechanisms for 'free' DNA and DNA c on the histone octamer.

- DNA is an extraordinarily non-linear optical material[12]. The nonlinearities are a strong function of counter-ion species, and appear to be associated with the phosphate groups[12]. We therefore might expect anomalous electronic properties as a consequence. Indeed early work on the Raman spectra in the 10-100 $cm^{-1}$ region appears to show most unusual behavior[13].

**3. The gigahertz dynamics of the hydration shell.**

DNA would be simple indeed if water could be treated as a classical viscous continuum, serving as a simple damping medium for vibrations of the double helix[14]. Such a simple picture would predict that the microwave resonances reported by Edwards et al.[1] should be overdamped[15]. Scott has proposed a soliton model to explain the lack of damping[16,17], but this requires that DNA have most extraordinary elastic properties[18]. It turns out to be very simple to probe the coupling between DNA and its hydration shell at GHz. frequencies usin Brillouin scattering. A simple account is given in the Appendix, and full report is being published[19]. The result of this investigation is a crude picture of the dynamics at a level just one step more complex than the simple continuous fluid, but with **important consequences for DNA dynamics at GHz. frequencies.** We find:

- The water at 'primary' hydration sites undergoes a viscoelastic transition at about 4 GHz. at physiological temperatures.

- The 'secondary' hydration undergoes this transition at about 80 GHz.

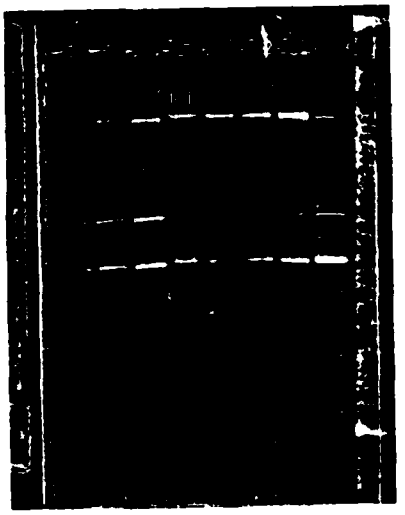

**Figure 1.** Agarose gel electrophoresis runs on the plasmid preparation - the wells were loaded with various concentrations to aid in the identification of trace contaminants. The bottom band is the intact plasmid, subsequent bands are relaxed plasmid and concatomers. The band nearest the well is probably genomic DNA.

Thus for motion well below 4 GHz., the water can indeed be treate as a viscous continuum which simply damps the motion. Above 80 GHz. all the water is elastically bound. That is, it stiffens and mass loads the vibration, but does not damp it because it can't follow it **In between these frequencies there lies a region of new excitations involving coupled motion of the DNA-hydration shell system.** These coupled motions provide a very simple explanation of the observed microwave resonances[20].

## 4. Search for direct evidence of standing wave modes on plasmids.

### 4.1 Motivation and samples.

The resonant microwave absorption experiments[1] are still a matter of some controversy, so an independent conformation of the existence of these modes in plasmids is important. The following two experiment could have confirmed the Edward's result. The results were negative. **Negative results in these experiments do not invalidate the Edwards data.** Because of the rather inconclusive nature of these experiments, we do not intend to publish descriptions, however we feel that the

work will be of interest to the other contractors in this program. It also suggests further experiments which we are anxious to carry out : we can organize a collaborative microwave-light scattering experiment

We describe two experiments here. One is a search for Raman signals from the plasmids, the other a search for acoustic coupling t the resonances.

A key point in this work is the condition of the sample. We grew pUC 8 plasmid in our laboratory from transvected e. coli supplied to us by Jeff Saffer of the Jackson Laboratory. The plasmid was amplifie with chloramphenicol, separated in the usual manner with RNase digestion and sepharose columns use to remove the ribonucleic acid components. A good fraction of a mg was obtain in each preparation (carried out by J. Powell when he was with our group). Agarose gels were run before and after each experiment to determine the condition of the plasmid (even small laser exposures appear to cause nicking ar relaxation). Figure 1 shows a typical gel. There is some relaxed material and a high molecular weight band we believe to come from contamination from e. coli genomic DNA (the method of preparation and characteristics of the gel make the band appear sharp). Nonetheless most of the material was intact plasmid.

**4.2 Search for Raman signals from the plasmid resonances.**

Although the frequency of the plasmid resonances lies way below the limit of most Raman monochromators, our tandem interferometer[21] i ideal for probing for weak excitations at gigahertz frequencies. We begin by asking what signal we might expect. Formally we can write th Raman scattering cross section (RSCS) as:

$$RSCS = C\ \omega^4\ |\varepsilon_S^i \varepsilon_I^j\ \chi^{ij}\ E_I^j\ |^2\ \ < X(q)\ X^*(q) >_\omega \qquad (1).$$

Where C is a constant that involves the concentration of the DNA. The term in straight brackets (||) is the product of the incident and scattered field polarization vectors with the appropriate polarizibility derivative and incident electric field. The last term in angle brackets is an average taken over the scattering volume of the square of the atomic displacements from equilibrium. Essentially we know the first two terms, since we know the strength of Brillouin signals from concentrated samples (and Brillouin scattering is, after all, just scattering from the acoustic phonons that, on an isolated plasmid, set up the acoustic resonances believed to be responsible fo the microwave absorption). The questionable parts are:

a) The concentration: Edwards et al. used samples of a few mg/ml, several hundred times more dilute than the fibers we used. We chose t do experiments at 1 mg/ml and 10 mg/ml. At the higher concentration, it is quite possible that aggregation could wash out the resonances.

b) The coherence problem: In Brillouin scattering, macroscopic soundwaves are studied. The atomic displacements in $< X X^* >$ are of course coherent, and the averaging over the spatially coherent part o this term leads to the well known "quasimomentum" conservation rule for Brillouin scattering:

$$k_I - k_S = q \qquad (2)$$

where q is the scattering vector

$$q = 2\,|k|\,\sin(\theta/2) \quad . \qquad (3)$$

Here $\theta$ is the scattering angle, and $k_I$ and $k_S$ are the incident and scattered wavevectors and $|k|$ is the magnitude of the wavevector of the light in the medium ($2\pi n/\lambda$). Equation 2 expresses the fact that, at a given scattering angle (the angle between $k_I$ and $k_S$), only one wavelength of phonon scatters coherently. The strength of this scattering will be proportional to $N^2$ where N is the number of atoms in the scattering volume. Other phonons and, in particular, other **incoherent** local modes scatter only through the incoherent fluctuation amplitude which varies as $\sqrt{N}$ giving a scattered intensity that varies as N. Thus **the ratio of scattering intensity between coherent (Brillouin) scattering and scattering from incoherent local modes is the same as the ratio of the Brillouin scattering to the Poisson noise on the Brillouin signal.** Thus, close to coherent Brillouin signals local mode signals could never be detected The key question is how coherent are the plasmid resonances (for the plasmid is of approximately the same size as an optical wavelength). This is a very hard question to answer. Suffice it to say that we have observed quite strong Raman signals from samples as dilute as 1 mg/ml, and therefore might hope to see something. We also beat the Poisson noise to some extent by carrying out the experiment with high digital resolution, and the digitally smoothing to the final required resolution

In the experiments we look at frequencies **above** the frequency of the Brillouin signal in backscattering : i.e. well above about 10 GHz This is because below that frequency, even the most obscure and tenuous stray reflection will cause a spurious Brillouin peak. We show a typical result in Figure 2. Here we have signal averaged for **four days**. The central feature is actually the bottom of the coherent Brillouin signal, here consisting of several million counts, and way off the scale! The peaks marked LAG are instrumental in origin. The peaks marked G are due to Brillouin scattering from the glass cuvette (even though no glass was visible to the intersecting beams!) while a number of small peaks (marked with arrows) appear to be real signal. Although these "real signals" are above the Poisson noise, we can make them appear in a similar experiment using calf thymus DNA! We believe that they are just an artefact produced by our digital smoothing algorithm.

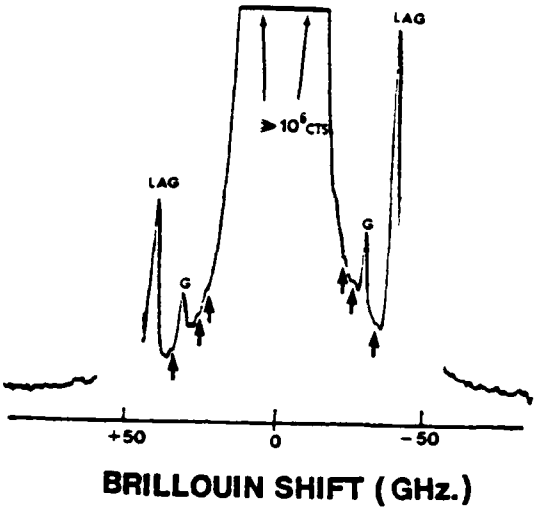


**Figure 2.** Typical light scattering spectrum obtained from a 1mg/ml plasmid solution after 4 days of signal averaging. The central part of the spectrum is the tail of the very strong coherent Brillouin scattering ( a pair of peaks centered at ± 9 GHz.). The other peaks are explained in the text. Instrumental ghosts at ± 50 GHz. have been removed.

**4.3 Search for acoustic coupling to plasmid resonances.**

Even if the Raman signals are weak (see above), it is possible that the acoustic phonons in the bulk solution might couple to the plasmid resonances. Whenever the bulk phonon frequency equals the frequency of a resonance, an increase in phonon linewidth might be seen as energy from the sound wave goes into mechanical pumping of the plasmid resonance. Thus one might expect to see absorption maxima on top of the classical $f^2$ absorption due to the viscosity of water. The Brillouin shift varies as

$$f_B = f_{B0} \sin(\theta/2)$$

where $f_{B0}$ is the Brillouin shift in backscattering (approximately 9 GHz. for a reasonably dilute DNA solution).

We performed 22 measurements at angles between 27° and 155°,

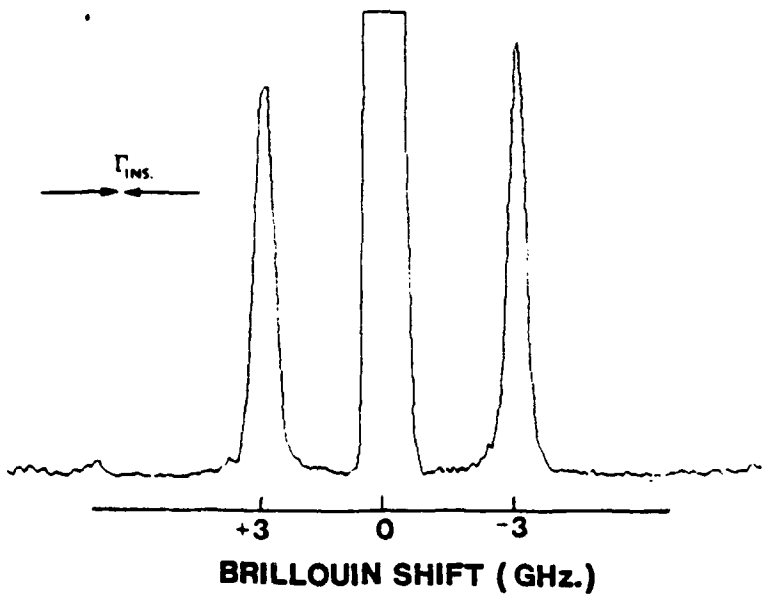


Figure 3. Typical Brillouin spectrum from 0.5 mg/ml plasmid solution showing Brillouin peaks at ± 3.14 GHz. The scattering angle was 36°. The instrumental fullwidth is about 150 MHz. and is illustrated by the arrows.

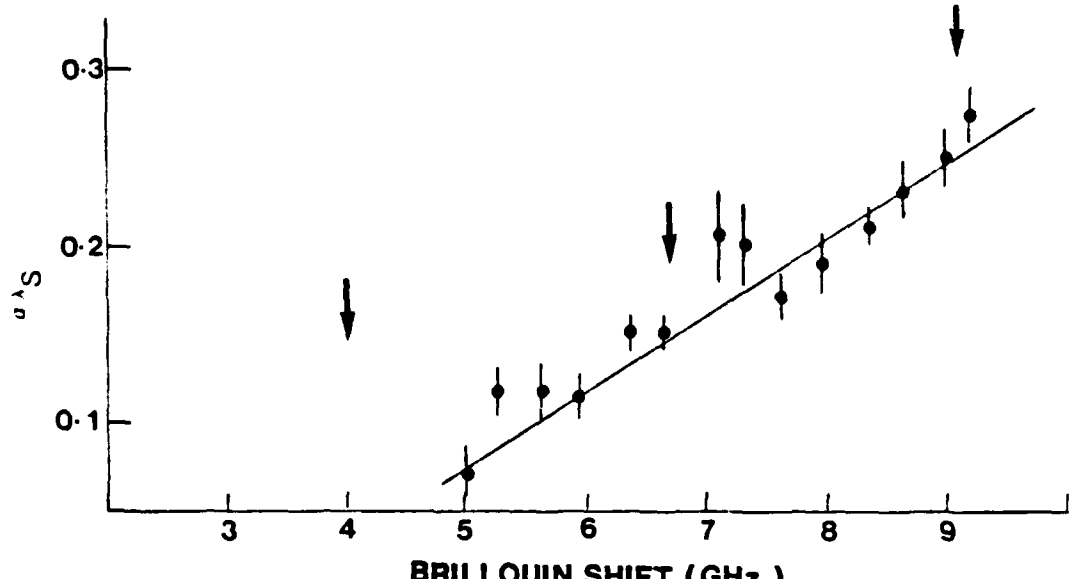


Figure 4. Normalized damping, $\alpha\lambda_S$ plotted with Brillouin shift. The arrows correspond to the frequencies of resonance reported by Edwards et al. The straight line is the classical $\alpha \sim f^2$ viscous loss.

covering Brillouin shifts from 2.56 to 9.18 GHz using a 0.5 mg/ml solution of pUC 8. We changed the scattering angle so as to give points that were equally spaced at about 500 MHz. intervals. Note that this frequency interval covers the region covered in the original experiments of Edwards et al.[1]. A typical spectrum is shown in Figure 3. We measure the linewidth, subtract the measured instrumental width and then correct for aperture broadening effects. Below about 5 GHz, the spectra are completely dominated by aperture broadening (the intrinsic linewidth varying as $f^2$). We obtain the dimensionless quantity $\alpha\lambda_S$, the product of the absorption coefficient and the phonon wavelength, from

$$\alpha\lambda_S = 2\pi \ \Gamma_{1/2}/f_B$$

where $\Gamma_{1/2}$ is the corrected HWHH of the Brillouin peak. $\alpha\lambda_S$ is proportional to f for a viscous loss process. In Figure 4, we plot $\alpha$ against frequency. The arrows mark the frequencies of resonances reported by Edwards et al. The straight line is the classical $\alpha \sim f^2$. Clearly any coupling to plasmid resonances is small

**4.4 Future experiment.**

We have demonstrated that any Raman signal from the plasmid resonances is too small to detect. This is almost certainly because such modes, thermally driven, are spatially incoherent. However, a key point about microwave pumping of the resonances is that it is a spatially coherent process. Thus providing that the wavevector of the illuminating field (q) is aligned according to equation 2, it ought to be possible to detect the resulting signal in the scattered light spectrum because it will be inherently as strong as the Brillouin signal, but reduced by some factor that reflects the concentration of plasmid. The success of such an experiment would demonstrate **coherent** pumping by the microwave field rather dramatically. This is because such low frequency modes are heavily thermally populated (a few thousand quanta per molecule) while the measured microwave absorption and lifetimes correspond to much less than one pumped quantum per molecule. In the absence of coherence effects, microwave pumping would have no effect on a scattered light spectrum.

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# LOW FREQUENCY COHERENT VIBRATIONS OF DNA: THE ROLE OF THE HYDRATION SHELL AND PHOSPHATE-PHOSPHATE INTERACTIONS.

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**Abstract.**

The vibrational modes of DNA span a range from high frequency localized vibrations, through low frequency collective modes to over-damped Brownian fluctuations. Presumably the most important motions from a biological standpoint are the lowest frequency vibrations (involving the largest units) that are not over-damped by the viscous action of the hydration shell. I describe observations of low frequency collective vibrational modes of DNA which couple to the hydration shell. The dynamics of the hydration shell becomes important in a frequency 'window' between the viscoelastic transition of the primary hydration shell (roughly 4 GHz.) and the viscoelastic transition of the secondary shell (roughly 80 GHz.). The role of coupled solvent-DNA dynamics in the A to B and B to Z transition is discussed in terms of the phosphate-phosphate interactions which probably dominate conformational stability. Excitations of coupled modes of the DNA-hydration shell system may also account for the resonant microwave absorption observed in restriction fragments and plasmids.

## 1. Introduction: The biological importance of gigahertz vibrations and the role of water.

Biopolymers are special in their complexity and the involvement of rather large 'molecular chunks' in their function. At the moment these components are understood phenomenologically. For example, subtle variations in 'static' structure can give clues about the molecular basis of such complex processes as recognition, but a full physical understanding would require knowledge of the movements of the atoms involved, and how these movements (phonons) interact with electronic states to bring about the binding and subsequent reaction. We can say with some confidence, however, that the interesting frequencies of motion must lie between a few GHz. and a few hundred GHz. No sophisticated analysis is needed - the upper end of the frequency range is set by the mass of the smallest 'bits' of molecule of biological importance (a basepair, for example) and the value of typical interatomic force constants[1]. The lower end of the time scale is fixed by the effects of the viscous drag of the

hydration shell. The time scale of heavily damped motion is characteristic of the viscosity of the damping medium and not details of atomic structure. Thus **the low frequency Brownian fluctuations cannot contribute to biology at the atomic level.** It is my purpose in this chapter to examine this low frequency transition from under- to overdamped motion, and to outline the importance of the hydration shell dynamics and 'structure' in conformational transitions and resonant microwave absorption.

I end this introduction by stressing that current theories of biopolymer motion ignore the hydration shell dynamics. Molecular dynamics simulations (which could handle the problem in principle) cannot yet reach gigahertz frequencies. As a final caution, I note that non-linearities can give rise to an important role for fluctuations on time scales many orders of magnitude slower than this in, for example, the nucleation of new phases[2].

## 2. The coupled dynamics of DNA and its hydration shell at GHz. frequencies.

### 2.1 Models of water dynamics

The simplest approach to the hydration shell is to treat it as continuous viscous water. The damping of a vibrational mode is then just a consequence of the velocity gradients set up in the surrounding water by DNA motion. In a dilute solution, all modes below a few tens of GHz. are overdamped[3]. This model predicts that acoustic standing wave vibrations at gigahertz frequencies must be overdamped, and cannot give rise to resonant microwave absorption[4]. However, some recent experiments appear to indicate that such modes are far from overdamped[5,6]. Could it be that water is in some way 'decoupled' from DNA? We have addressed this question with Brillouin scattering studies of DNA films at various degrees of hydration and as a function of temperature[10].

### 2.2 Determination of hydration shell relaxation by Brillouin scattering.

In a Brillouin scattering experiment, light is inelastically scattered by an acoustic phonon (sound wave). The wavelength of the scattering phonon depends on the scattering geometry[7], so the well known linear dispersion of sound waves results in a Brillouin signal shifted in frequency by anything from a GHz. to a few tens of GHz., depending on the speed of sound and the scattering angle (the wavelengths of the phonon involved is of the same order as the wavelength of visible light). The damping of these phonons (measured through the linewidth of the Brillouin signal) is both a strong function of the water content of fibers[8,9] and the temperature in a way that can be accounted for by the coupling of the acoustic phonon to two relaxation processes in the surrounding water[10]. We confirm this elementary analysis by independent measurements in which the phonon frequency is varied at a fixed temperature, and by theoretical models of the overall spectral profile[10]. A summary of our initial measurements is shown in Figure 1 where we plot the natural logarithm of the relaxation time (the units are seconds per radian) with reciprocal temperature for a sample with 'primary' hydration ( approximately 0.5 gm of water per gm of DNA) and a sample with 'secondary' hydration' (approximately 1.5 gm of water per gm of DNA). Overall, the 'primary' relaxation

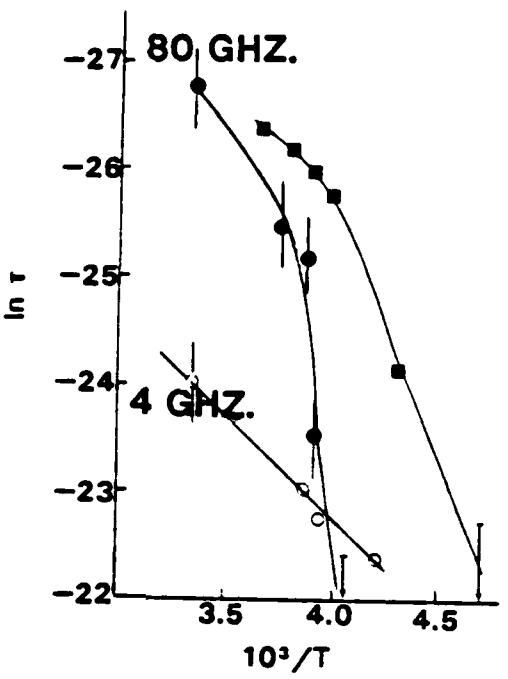


**Figure 1.** Plot of the natural logarithm of relaxation time with reciprocal temperature for a sample with primary hydration (○), and a sample with secondary hydration (●) as measured by Brillouin scattering. The remaining data (■) are for a sample with secondary hydration and are extracted from the Raman experiments of Tominaga et. al.[11]. Times are in seconds per radian - corresponding frequencies are marked in GHz.

strength appears to increase as water is added up to about 1gm water per gm of DNA[9]. At higher water contents, a fast process associated with the 'secondary' shell dominates (though the slow primary process appears to persist).

**2.3 Implications of the measured hydration shell relaxation.**
The two most important implications are:

i)GHz. acoustic modes are strongly coupled to the hydration shell.

ii) The structure of the hydration shell leads to specific dynamical excitations associated with that structure - 'bound' water cannot be treated as a uniform viscous medium at these frequencies. The 'primary' shell undergoes a viscoelastic transition at about 4 GHz. at physiological temperatures. The 'secondary' shell undergoes this transition at about 80GHz.

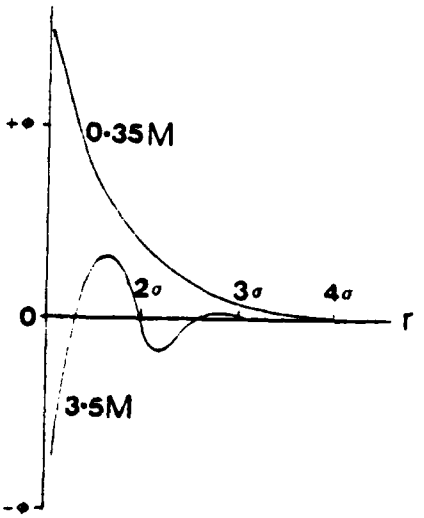


**Figure 2.** Effective anion-anion interaction potential for low (upper curve) and high (lower curve) salt concentrations. $\sigma$ is the distance of closest approach of a hydrated ion pair. Note the appearance of new attractive regions at high concentrations.

The simplest possible modification of the 'uniform' hydration shell picture is this: Below about 4GHz. all the water surrounding the double helix is viscously coupled, so on the whole, modes below this frequency will be overdamped. Above about 80 GHz. all the water is elastically coupled so the water will effectively stiffen (but not damp) motion. In the intermediate region, **there are important extra degrees of freedom associated with the coupled dynamics of the DNA-primary hydration system.** In other words, **in exactly the region of most biological importance, water plays an explicit dynamical role.**

The static structure of the hydration shell is hinted at in several experiments[12,13,21] and also by Monte Carlo calculations[14]. Furthermore the electrolyte surrounding the double helix plays a critical role in conformational stability[15,16]. We therefore expect the coupled motions of the double helix and hydration shell to be of great importance.

## 3. Water dynamics, conformational transitions and interactions between phosphates.

### 3.1 Phosphate-phosphate interactions and conformational stability.

Soumpasis[15,16] has identified the key role played by phosphate-phosphate interactions (mediated by the surrounding electrolyte) in conformational stability. The negative charges are treated as point anions interacting via the electrolyte through an effective pairwise interaction (calculated from a true many-body model based on ion-ion correlation functions). At low salt concentrations, this effective potential is everywhere

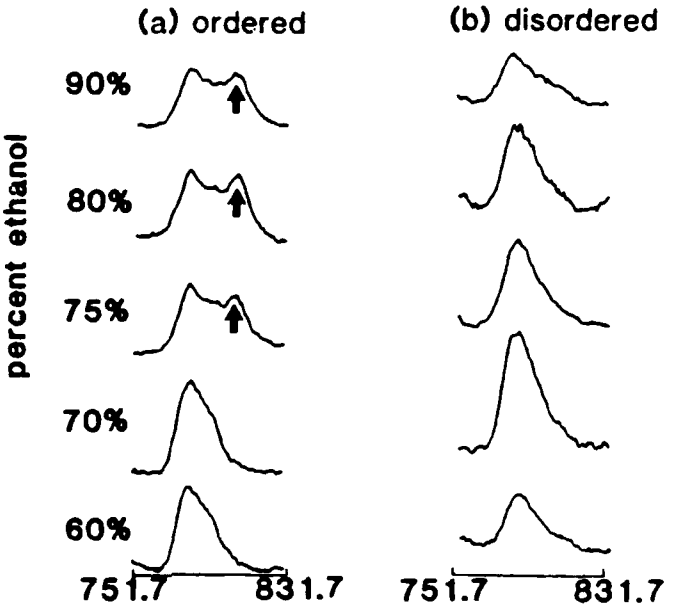


Figure 3. Raman spectra showing (a) the appearance of the 807 $cm^{-1}$ A form marker band (indicated by an arrow) in crystalline samples as ethanol displaces water (percentages marked next to the spectra) and (b) the lack of the A form marker band as the amorphous samples are dehydrated (abscissa are Raman shifts in $cm^{-1}$).

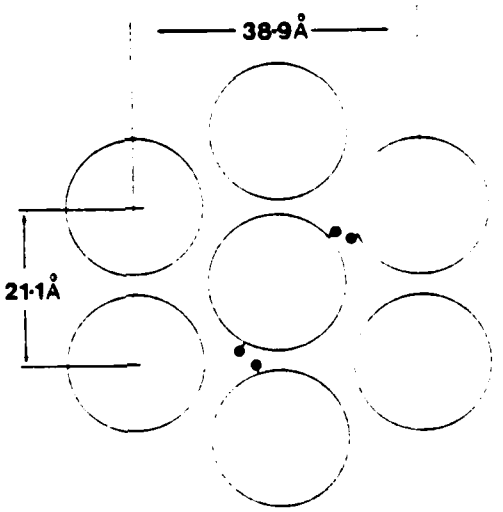


Figure 4. View of the interhelical contacts in A-DNA looking down into the ab plane of the monoclinic unit cell (dimensions are for 54% r.h.). The full circles correspond to the P-P diameter of the double helices and the black dots show the location of the O-2 atoms that come within 3.5Å of each other (for the central molecule only). The breaking of the apparent 6-fold symmetry is a consequence of the incomensurable nature of the 11-fold helix with the nearly 6-fold packing geometry.

repulsive, falling off in the familiar Debye-Hückel manner expected for screened Coulomb interactions. At high salt concentrations, however, the interaction is changed dramatically. It is not at all monotonic, and attractive in places. This structure reflects the details of the ion-ion correlation functions. Figure 2 shows this effective anion-anion interaction as calculated by Soumpasis[16] for a 1:1 electrolyte of 0.35 and 3.5 M concentration. Note, in particular, the complete reversal near the origin! The length scale is parameterized in terms of the distance of closest approach of the hydrated ions, σ. This parameter may vary between about 3Å and 8Å, but fits to experimental data are obtained with σ close to 5Å[15,16]. I should emphasize that the theory assumes an electrolyte solution, so that features in the high salt effective potential do not correspond to fixed charge structures. In discussing transitions that occur as solid DNA is diluted, I will outline possible charge structures with the understanding that they may be static, or represent some time average over fluctuating structures.

**3.2 Interhelical interactions and conformation transitions.**

The theory of Soumpasis has proved remarkably accurate in describing the salt dependence of the B-Z transition[15]. It is less satisfactory in describing other transitions[16]. However, as we have shown elsewhere[17], the A to B transition appears to be driven by solid state interactions. (This does not appear to be the case for the B-Z transition[18]). We provide a further demonstration of the effect of crystallinity on the A-B transition in Figure 3 which shows Raman spectra obtained from crystalline and amorphous films as water is displaced by ethanol[19]. The 807 $cm^{-1}$ 'A-form marker band' appears only in the crystalline sample, demonstrating that aligned strands are needed to form A-DNA. Brillouin scattering experiments show that there are strong interactions between adjacent helices in the solid state[9,17]. So strong are these bonds, that highly crystalline films can be nearly insoluble[20]. A-DNA is more closely packed than B-DNA, so the structural fluctuations due to sequence heterogeneity are smaller[21], and thus A-DNA should be capable of forming a more regular pattern of interhelical contacts in the solid.

The nature of the interhelical contacts in the crystalline regions can be obtained from a study of the unit cell geometry. In A-DNA the double helices pack into a monoclinic cell with (at 54%r.h.) a=21.1Å, b=38.9Å, c=27.3Å, β=97° and an additional double helix at (1/2, 1/2, 0)[17]. Although the closest approach (22.1Å center to center) brings the double helices almost into contact (at their O-2 radius), the three dimensional packing minimizes O-2 clashes, with the backbones of one double helix lying in the major grooves of its neighbors on the whole. However, geometry does not permit the three dimensional interlocking of screws of the same handedness and clashes do occur. These are illustrated (for the central molecule only) in Figure 4 which shows a view down onto the unit cell. The circles correspond to the P-P diameter (nearly 18Å) and the black dots show the location (in the ab plane) of the O-2 atoms in one turn of the central molecule that come within 3.5Å of O-2 atoms on adjacent double helices. There are two such shared clashes per turn (i.e. one per double helix per turn), and it is interesting to note that the 'magic' amount of excess NaCl (1% by weight) required to give good crystalline x-ray patterns gives almost exactly one extra $Na^+$ per double helical turn.

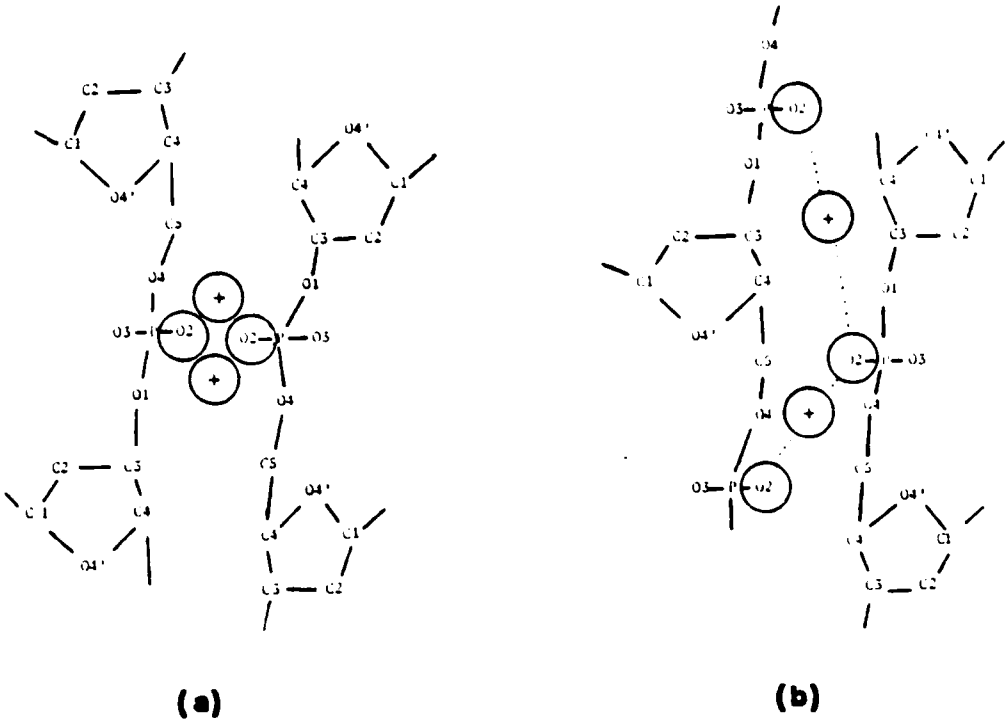


Figure 5. Some possible effective charge distributions for (a) close O-2 interactions at backbone clashes (the O-2 to O-2 separation is typically 3.3Å) and (b) for interactions where the O-2 of one molecule lies in the major groove of its neighbor (separations range from about 8 to 12Å).

The O-2 interactions fall into two categories: the close approaches at backbone clashes, and the more common interactions as the backbone of one molecule packs into the major groove of another. The structures are sketched in Figure 5. It is quite possible that many of the O-2 interactions fall into the attractive regions at $1\sigma$ and $2\sigma$ in the high salt potential (Figure 2). The strength of the 'electrolyte' is indeed in the molar region for fairly dry (A-DNA) fibers, but of course the applicability of a liquid electrolyte model must be somewhat questionable. A system of attractive interactions could be set up with positive charges distributed as indicated in Figure 5. The closest approaches (5a) do not leave room for a hydrated cation between the oxygens, but nonetheless, if the potential of Figure 2 applies, these may form the strongest interhelical bonds.

Whatever the detailed structure of these clusters, they presumably form some alternating array of charge held together by the Coulomb interaction. A feature of this interaction is its rapid variation near the origin. In this regard, 'cartoons' like Figure 5 are rather misleading. The simple arrangement of charge

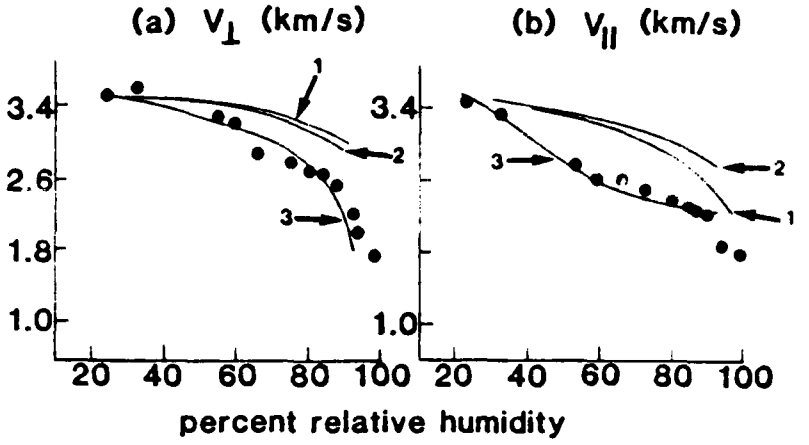


**Figure 6.** Softening of the sound speed as a function of relative humidity in Na-DNA films (a) perpendicular to the helix axis and (b) along the helix axis. Curve 1 is calculated for mass loading, curve 2 for relaxation and curve 3 for Coulomb softening of the phosphate interactions.

in 5b is more likely to give an $r^{-1}$ potential than the more complex close packed structure illustrated in 5a. Note, however, that the high salt potential (Figure 2) is much more like a hyperbola at $1\sigma$, while the minimum at $2\sigma$ is much more like a harmonic ($r^2$) potential (in reality, the phosphate interactions are probably yet more complicated due to the very large polarizibility derivatives associated with the phosphate groups[22]). We have considered a number of charge geometries and screening effects[9], and conclude that, for small displacements of the interacting anions (see ref. 17), a Coulomb interaction would almost invariably appear to fall off in a manner close to the classic $r^{-1}$ behavior initially. In consequence, if the O-2 atoms move apart as the center to center separation of neighboring double helices,d , is increased, the interhelical force constants should fall off as

$$c_{ij} = k\, d^{-3} \quad .$$

We measure d by fiber diffraction, so we can test for such Coulomb interactions by comparing Brillouin measurements of the speed of sound with the predictions of a lattice dynamical model in which the interhelical springs are softened as $d^{-3}$. I should make it clear that the sound speed is a particularly sensitive probe of these long range interactions. Of course these long range interactions must be combined with other short range interactions to form a stable structure, but long wavelength sound waves are not sensitive to short range interactions. Figure 6 shows Brillouin data for the speed of sound perpendicular (a)

and parallel (b) to the axis of Na-DNA films as a function of relative humidity (over most of this range the swelling of the crystal is indeed small[17]). The sound speed might also be affected by mass-loading as the water binds, and relaxational softening as water motion becomes more free. Water is relaxationally coupled in this frequency region[10] - for this reason we can exclude mass loading (which would have a small effect anyway). The relaxation strength is not enough to account for much of the softening. On the other hand, if we fit the scale (k) of the interhelical interactions to match the sound speeds at low water contents, the Coulomb softening theory outlined above fits the data remarkably well (curve 3 on Figures 6a and 6b), demonstrating the dominant role of these interactions.

**3.3 Collective excitations and conformation transitions.**

In the above picture of the A-B transition, the mechanism might be understood in terms of a crossover to simple screened Coulomb repulsion as the cation cloud is diluted by water addition. Much more must be known about the structure of the hydration shell before a dynamical pathway for the transition can be constructed. However, it is clear that such a pathway **must involve collective excitations of DNA and its hydration shell.** This point is particularly important for the B-Z transition, for the mechanisms that have been proposed (based on 'dry' helix models) involve substantial energy barriers[23,24]. It is possible that a low energy pathway can be constructed with one or more collective modes of the polymer and its hydration/electrolyte shell.

**4. Microwave resonances.**

The length dependent microwave absorption reported by Edwards et al.[6] is quite remarkable. The simplest interpretation of the absorption maxima is that they correspond to the frequencies of acoustic standing waves on the DNA. The frequency of such waves will be some multiple of the speed of sound, V, divided by the length of the fragment, L. DNA itself lacks any large dipole moments, but (so the argument goes) the counter ion cloud cannot follow GHz. motions so that the relative compressional displacement of the phosphates sets up an oscillating dipole with respect to the 'static' counter ions. The microwave field is essentially homogeneous on a molecular scale, so alternating positive and negative dipoles cancel each other out. An odd number of half wavelengths is needed to absorb energy on a linear fragment. Putting all this together gives the formula that fits the observed absorption peak frequencies quite well:

$$f_n = (n+1/2)\ V/L \quad .$$

The speed of compressional sound waves, V, obtained by fitting the microwave data, is in close agreement with the value obtained from Brillouin scattering measurements[25]. The remarkable thing about this interpretation is the coherence length it implies for acoustic phonons. These plasmid fragments are a good fraction of a micron in length. In order to build up standing wave amplitude, the vibration must be capable of many passes up and down the polymer. There would be important biological consequences of such vibrational coherence lengths, but it is hard to see how the phonon damping could be so small. Scott has made the novel

suggestion that DNA is such an (elastically) non-linear material, that compressional waves travel as Boussinesq solitons[26,27]. These solitons are familiar as shallow water waves (Scott often illustrates his talks with a glass dish full of water). Shallow water is extremely non-linear in the sense that the speed of waves is a strong function of their amplitude (and by 'shallow' we mean that the depth is of the same order as the wavelength ). Thus crests of waves catch up with troughs, and the water travels in 'bunches' rather than being distributed in sinusiodal waveforms. The point about such solitons is that the moving part of the wave becomes localized. The more non linear and localized the excitation, the more energy goes into the 'static' distortion part of the soliton (i.e. the stored spring energy). Consequently, the viscous losses are reduced as the moving area of the polymer in contact with the water is reduced. The key question is whether DNA is as elastically non-linear as shallow water. I share the the reservations of Van Zandt[28]. Thermal amplitudes are very small - a tiny fraction of bond lengths, and while non-linearities may make themselves felt, it is hard to see how they could dominate to the extent required to explain the lack of viscous damping[27]. As an aside, it is worth noting that the microwave absorption signal corresponds to less that one pumped quantum of vibration per molecule - thermal energy corresponds to several thousand quanta at these frequencies. The thermal excitation is incoherent, so the amplitudes of the vibrations do not add. The displacement is always a small fraction of the bondlength.

The dynamical behavior of the hydration shell may offer an alternative explanation of these sharp resonances. As I pointed out at the beginning of this contribution, there are collective excitations of the DNA and the hydration shell in just this frequency region. Excitations of this sort (coupled to compressional phonons on the DNA) could account for the observed resonances[29].

## 5. Conclusions and summary.

The hydration shell and counterion cloud, interacting with the DNA via the charged phosphates, play an important role in the biologically important dynamics and in the conformational stability of the double helix. Coupled DNA-hydration shell excitations may account for resonant microwave absorption. Theories of the most important vibrational modes of the double helix must include excitations of the coupled DNA-water-ion system as well as explicit phosphate-phosphate interactions mediated by the electrolyte. These interactions have, on the whole, been ignored as dynamical studies focus on the covalent bonds that make up the Watson Crick structure. For many problems, it may be that the only importance of the covalent structure is the role it plays in locating charges in space - the dynamics being dominated by the interactions of this charge system with the electrolyte and, through the electrolyte, with itself.

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0487)) and EPA (68-02-4105 - this chapter was not subject to EPA review and does not necessarily reflect the agencies views).

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