



Solvent effects on the electronic spectra of indoles : theoretical methods and laser induced fluorescence excitation in supersonic jet
by Pedro Luis Muino Montero

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University
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Abstract:

The effect of solvent on the first two $\pi \leftarrow \pi$ excited states of indole and 3-methylindole, (1La and 1Lb), has been studied using a combination of hybrid theoretical methods and experimental techniques. A procedure that couples molecular dynamics with semiempirical molecular orbital methods has yielded information about the mechanism and time scales involved in bulk solvent reorganization after excitation of the indole molecule to the 1La, (or 1Lb), state. The fluorescence red shifts predicted in several solvents, (water, butanol, methanol and dimethylether), agree reasonably well with experimental values. These time resolved calculations also indicate that the solvent relaxation has two components: The first one is inertial in character, with a Gaussian shape having a half-width at half-maximum of ~ 15 fs for water, and of 100-300 fs for the other, (larger), solvents. The second component shows an exponential decay behavior and seems related to the longitudinal relaxation time of the solvent. The correlation times for this component are ~ 170 fs for water, and a few picoseconds for the larger solvents. By coupling molecular mechanics with semiempirical molecular orbital methods, information was obtained about the relative populations and spectroscopic properties of the different conformers of tryptophan at 300 K. We also present very detailed one-photon excitation spectra of indole complexed with H₂O, D₂O, and methanol, along with an analysis of the intermolecular vibrational modes of the two types of complexes present in each spectrum. The two-photon excitation spectra of selected peaks helped in determining the 1Lb or 1La character of a few transitions. The origin of the first of the complexes has been assigned as 1Lb and the putative origin of the 1La manifold has a polarization ratio compatible with 1La character. Analysis of the ring vibrational modes associated with the other complex suggests that its origin also has 1Lb character.

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THEORETICAL METHODS AND LASER INDUCED FLUORESCENCE
EXCITATION IN SUPERSONIC JET

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Pedro Luis Muiño Montero

Advisor: Dr. Patrik R. Callis

Montana State University
1993

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APPROVAL

of a thesis submitted by

Pedro Luis Muiño Montero

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

16 Nov 93

Date

Patrick R. Callis

Chairperson, Graduate Committee

Approved for the Major Department

Nov. 16, 1993

Date

David M. Foley

Head, Major Department

Approved for the College of Graduate Studies

11/21/93

Date

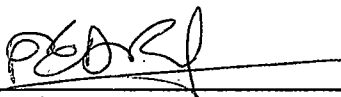
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To those closest to me: mamá, papá, Toni, madriña, padriño, and Penny.

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ABSTRACT

The effect of solvent on the first two $\pi^* \leftarrow \pi$ excited states of indole and 3-methylindole, (1L_a and 1L_b), has been studied using a combination of hybrid theoretical methods and experimental techniques. A procedure that couples molecular dynamics with semiempirical molecular orbital methods has yielded information about the mechanism and time scales involved in bulk solvent reorganization after excitation of the indole molecule to the 1L_a , (or 1L_b), state. The fluorescence red shifts predicted in several solvents, (water, butanol, methanol and dimethylether), agree reasonably well with experimental values. These time resolved calculations also indicate that the solvent relaxation has two components: The first one is inertial in character, with a Gaussian shape having a half-width at half-maximum of ~ 15 fs for water, and of 100-300 fs for the other, (larger), solvents. The second component shows an exponential decay behavior and seems related to the longitudinal relaxation time of the solvent. The correlation times for this component are ~ 170 fs for water, and a few picoseconds for the larger solvents. By coupling molecular mechanics with semiempirical molecular orbital methods, information was obtained about the relative populations and spectroscopic properties of the different conformers of tryptophan at 300 K. We also present very detailed one-photon excitation spectra of indole complexed with H_2O , D_2O , and methanol, along with an analysis of the intermolecular vibrational modes of the two types of complexes present in each spectrum. The two-photon excitation spectra of selected peaks helped in determining the 1L_b or 1L_a character of a few transitions. The origin of the first of the complexes has been assigned as 1L_b and the putative origin of the 1L_a manifold has a polarization ratio compatible with 1L_a character. Analysis of the ring vibrational modes associated with the other complex suggests that its origin also has 1L_b character.

INTRODUCTION

The indole molecule has been studied¹⁻⁷ in detail in order to get information about the amino acid tryptophan, (see Figure 1), which is the main contributor to the UV absorption and fluorescence of proteins^{4,8-10}. The spectrum of indole and derivatives, however, is complicated by the presence of two near degenerate $\pi^* \leftarrow \pi$ transitions¹¹ labelled 1L_a and 1L_b ¹².

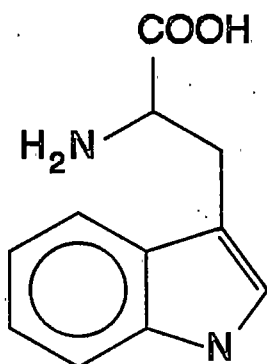


Figure 1. Tryptophan

The work of Valeur and Weber⁶, in 1977, was successful in applying fluorescence polarization techniques to separate the 1L_a and 1L_b contributions to the excitation spectrum of indole in propylene glycol at 215 K. As a result of this work, more information about the nature of these two states was obtained: the 1L_a state presents a broad, featureless spectrum, possibly because of a Franck-Condon progression stemming from a geometry displaced from that of the ground state; while the 1L_b state results in a

sharp band as a result of a geometry similar to that of the ground state.

Early studies by Strickland *et al.*⁴ suggested that the nature of the solvent, and substitution at the 3 position, (see Figure 2 for numbering of the ring), affected the shape of the spectra. More thorough studies indicated that indeed substitution^{4,8,9,13}, solvent effects^{4,8,9,13-15}, and charge effects¹⁶ affect the 1L_a and 1L_b states differently, thus leading to changes in the spectra.

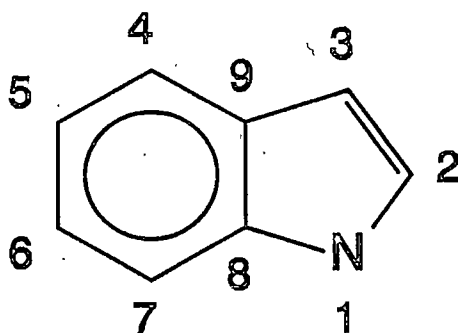


Figure 2. Numbering of Indole

Therefore, it was understood that differences in the electronic spectra of proteins could be analyzed in order to get information on the environment surrounding tryptophan inside a protein^{14,17,18,19,20}, yielding information about its tertiary structure. A better understanding of the indole chromophore is necessary to accomplish this task. In this respect, Lautié *et al.*⁷ presented the first infrared and Raman spectra of indole, which resulted in a complete study of its ground state vibrational frequencies. Lami and Glasser¹³ performed a very detailed analysis of the effect of substitution and solvent on the 1L_a and 1L_b absorption and 1L_a fluorescence shifts.

When the technique of laser excitation in supersonic jet was first applied to

indole²¹ and to indole+solvent^{22,23} it was clear that it would prove useful to get information about the vibrational frequencies of the excited²⁴⁻²⁷ and ground states^{27,28} for different indole derivatives. However, through this period, a very important question, that of the location of the 1L_a origin of indole, remained unanswered. Wallace *et al.*^{27,29} and Sulkes *et al.*³⁰ suggested that the 1L_a transitions could not be seen in supersonic jet because this state is dissociative, leading to a breaking (probably) of the N-H bond. It is possible, however, that the 1L_a origin was mistakenly identified as a 1L_b vibrational mode, (or combination of), because the fluorescence lifetime would be similar to those typical of the 1L_b state. The fact that indoles present normally single state emission³¹ can be responsible for this behavior, since the collected fluorescence would come from S_1 , independently of which state was excited.

On a parallel course, Callis *et al.* had been successful in applying the technique of two-photon spectroscopy to resolve the 1L_a and 1L_b bands of indole and benzimidazole³², methylindoles³³, and other aromatic systems³⁴. In 1990, this technique was used in combination with a supersonic expansion for indole³⁵, and succeeded in yielding the first positive identification of 1L_a transitions in jet. Callis *et al.* reported that the transitions at 455 and 480 cm^{-1} to the blue of the 1L_b 0-0 have 1L_a character. As the spacing between them, $\sim 25 \text{ cm}^{-1}$, was too small to correspond to a vibrational mode of the 1L_a state, it was suggested that the 1L_a origin is split, possibly as the result of a coupling with a nearby dark 1L_b state.

The effect of solvent dynamics has also been subject to a detailed study during the past years. Specifically, it is now well understood how the solvent affects electron transfer reactions³⁶⁻³⁸. Fleming *et al.*³⁹⁻⁴¹ and Barbara *et al.*^{42,43} have pioneered work

on subpicosecond spectroscopy which has resulted in a much better understanding of molecular phenomena and solvent reorganization at time scales as short as 50 fs⁴⁴ for coumarin dyes in water. These experiments have been coupled to numerous calculations on the behavior of different molecules in polar solvents⁴⁵⁻⁵¹. This work has helped in establishing that the temporal response of the solvent is dominated by solvent properties, but the magnitude of the shift clearly depends on the difference of the permanent dipoles of ground and excited states⁵¹.

It should also be pointed out that self consistent reaction field methods are appearing as of lately⁵²⁻⁵⁵. In these methods, the solvent is modelled as a dielectric continuum. They are useful for predicting, in an easy way, equilibrium solvation energies. However, they cannot predict good spectroscopic information for different solvents because the dielectric continuum formulation is not sensitive to changes in dielectric constant when $\epsilon > 10$, (see RESULTS AND DISCUSSION). Lami and Glasser¹³ have measured different shifts for indoles in five different solvents with dielectric constant larger than 10, (ranging from 17 to 80). They found that each solvent induced a different shift, contrary to what the self consistent reaction field methods would predict.

The question of which mechanism is responsible for the red shift induced by solvation has often been addressed in the literature. A very interesting theory explains the fluorescence red shift by means of *exciplex* formation. Exciplexes are excited-state solute-solvent complexes. Lumry *et al.*^{2,5} suggested that exciplex formation was responsible for a large red shift and a loss of vibrational structure in the fluorescence spectra of indole in polar solvents. They reported a solute-solvent stoichiometry of 1:2 and 1:1 for associating and non-associating solvents and dismissed the possibility of

hydrogen bonding between the N-H group and the solvent. One important characteristic of this work is that the spectra were taken in indole solutions in non-polar solvents containing small amounts of polar solvents. Thus, it is conceivable that the formation of the exciplex was induced by the presence of the non-polar environment which would tend to enhance the degree of association of the polar components.

The idea of exciplex formation has been used by Tubergen and Levy²⁶ and Sulkes and Arnold³⁰ to explain the broad and red shifted emission of the second of the complexes that indole forms with water in a supersonic jet. They have stated that the first complex is hydrogen bonded to the N-H group, while the second is located over the π cloud of the ring, producing an effect similar to the one seen in solution². The lifetime results of the fluorescence taken by Sulkes and Arnold are compatible with the idea that a second state is emitting. This would indicate that, upon complexation, the 1L_a state origin becomes nearly degenerate with the 1L_b origin and emission from both states follows. Presumably, the broad, red shifted emission would be caused by the 1L_a state.

Statement of the Problem

Our goal in this research is to shed some light on the problem of whether the assigned 1L_a origin is truly 1L_a or is a pair of 1L_b transitions borrowing 1L_a character through Herzberg-Teller coupling. We will also investigate the effect of the solvent on the ground and electronically excited states. To this end, we will be using two approaches which are going to yield information about two different aspects of the phenomenon.

Molecular dynamics coupled with semiempirical molecular orbital methods can yield good results about the behavior of indole in a bulk solution. The time scales involved in molecular dynamics, (femtoseconds to picoseconds), allow one to follow the dynamics of the indole molecule in the instants following excitation, when the solvent molecules are responding to the changes in the electric field generated by the solute. This will allow us to predict fluorescence shifts in solution. Comparison of results in different solvents will also provide information about the effect of the polarity of the environment and the size of the solvent molecules. Direct inspection of the solvent reorientation in response to the changing electric field generated by the solute will be very useful in helping to determine the mechanism responsible for the red shift.

On the other hand, we will apply the techniques of one- and two-photon laser induced fluorescence in supersonic jet to obtain information on how a single solvent molecule, forming a van der Waals complex with indole, affects the electronic states. We can use one-photon spectroscopy to get a very detailed picture of the vibrational frequencies, (both intra- and intermolecular), of the complexes using different solvents, and two-photon spectroscopy to obtain information on the nature of some of the transitions in the electronic spectra. This information can be coupled to that obtained by analysis of the differential shifts on the 1L_a and 1L_b peaks induced by complexation.

Comparison of these two techniques, which represent the many molecules as well as the single molecule limit, will provide information that will help in answering the questions of how, in which way, how fast, and how much the solvent molecules affect the indole chromophore.

BACKGROUND

Theory

This section contains a quick overview of the methods we are going to use in our calculations. It is our intention to explain their purpose, advantages and drawbacks, without giving an in-depth explanation of the theory behind them.

Molecular Orbital Methods

In general, quantum mechanics methods try to solve the Schrödinger equation, (see Equation 1), in order to obtain the associated eigenvalues and eigenfunctions.

$$\hat{H}\Psi_n = E_n \Psi_n \quad (1)$$

However, when three or more particles, (including nuclei and electrons), are present, Equation 1 does not have an analytical solution, and approximate methods are necessary. The reason for this impossibility to solve the equation stems from the presence of electron repulsion terms, (of the form of $1/r_{12}$), in the Hamiltonian of the system.

One way to write a one-electron Hamiltonian which includes the repulsion between that electron and the others consists of explicitly using the one-electron wavefunctions when building $\hat{H}$. However, this introduces the problem of having to know $\hat{H}$ to get Ψ , and having to know Ψ in order to have $\hat{H}$. To get around this situation, the best way to proceed is to start with a *guess* wavefunction, $\psi^{(0)}$, and use it to construct an approximate

Hamiltonian, $\hat{H}^{(0)}$. Now, we can solve the Schrödinger equation and obtain a better wavefunction, $\psi^{(1)}$, which is used to obtain a better Hamiltonian, $\hat{H}^{(1)}$. This process is repeated until self-consistency, that is, until the wavefunction obtained from the Schrödinger equation is the same that was used to build the Hamiltonian, (that is, $\psi^{(n)} = \psi^{(n+1)}$). Obviously, the better $\psi^{(0)}$, the faster the method will converge.

The wavefunctions obtained and used in the Self Consistent Field (SCF) method are multielectron wavefunctions and, in their simplest form, can be thought of as products of one-electron spin-orbitals⁵⁶. However, one of the postulates of Quantum Mechanics states that an acceptable wavefunction must be antisymmetric with respect to the exchange of electrons. To this effect, Slater⁵⁷ showed that one can assure antisymmetry with respect to the exchange of electrons by writing the wavefunction as a determinant whose elements are one-electron spin-orbitals. For instance, the multielectron wavefunction for the helium atom in its ground state can be expressed as

$$\Psi = \frac{1}{\sqrt{2}} \begin{vmatrix} 1s(1)\alpha(1) & 1s(2)\alpha(2) \\ 1s(1)\beta(1) & 1s(2)\beta(2) \end{vmatrix} \quad (2)$$

The SCF method cannot reach the true energy of the system because the electronic motion is not correlated. Each electron is seeing the other electrons as a time-averaged charge cloud, but there is no penalty built in the wavefunction preventing all the electrons from simultaneously occupying the same point in space. The minimum energy that can be obtained without including electron correlation is called the Hartree-Fock limit. Thus, a Slater determinant is an approximate solution of the Schrödinger equation, but it cannot

be used, by itself, to reach the true energy of the system.

To go beyond this limit, one has to include electron correlation. There are several ways to do so. The method most widely used is called Configuration Interaction, (CI). In this method, the wavefunctions are built as linear combinations of Slater determinants corresponding to different configuration of the system, (see Equation 3),

$$\Psi = \sum_i c_i D_i \quad (3)$$

where the D_i s are Slater determinants.

The configurations previously mentioned correspond to the ground state of the molecule, (1 configuration), and as many excited state configurations as one desires. The latter ones correspond to promotion of one electron to an unoccupied orbital, (singly excited configuration), of two electrons to two unoccupied orbitals, (doubly excited configuration), et cetera.

With the inclusion of CI, it is possible to reach the exact energy limit. The difference between this and the Hartree-Fock limit is called the correlation energy, and it is rather large. For instance, for helium, it is on the order of 25 kcal/mol⁵⁸. In our calculations, we are going to include CI, because it helps in better reproducing the spacing between the 1L_a and 1L_b states of indole, as shown by Callis⁵⁹.

Approximate Methods: INDO/S

With the use of computers, every integral stemming from the Schrödinger equation can be computed, even for fairly large systems. This is the basis for the *ab initio*

methods. These, however, are computationally intensive, because the number of integrals to be evaluated grows as the fourth power of the number of the basis function used.

If the molecule is large, the number of integrals extended over four nuclei is very large. Clementi and Mehl⁶⁰ have shown that many of these four center integrals are almost negligible. It would be interesting to devise a method that allows one to selectively neglect these integrals without having to spend time in calculating them. There are several considerations to take care of. For instance, if all the integrals neglected were very small, but all of them added together had a fairly large value, it would not be very wise to neglect them. Another problem to be addressed is that of relating the process of neglecting integrals to the basis function in a simple way. If one is not careful, different energies can be obtained for a molecule if its orientation changes.

Pople and coworkers⁶¹⁻⁶⁵ have developed a series of methods that take care of these and other problems and yield good results when calculating different molecular properties. In the first paper of the series⁶¹, Pople *et al.* introduced 5 approximations in the process of calculating the integrals. This method, called CNDO, (Complete Neglect of Differential Overlap), is the simplest of a group of methods based on the concept of Zero Differential Overlap, (see reference 56). Normally, these methods are also parametrized, indicating that scaling factors or parameters are included in the program so it yields good results for certain molecular properties.

The method we are going to use in our calculations is called INDO/S, (Intermediate Neglect of Differential Overlap, calibrated for Spectroscopy), due to Zerner *et al.*⁶⁶⁻⁶⁹. Ridley and Zerner⁶⁶ preferred the INDO over the CNDO formulation because

the latter does not include the one-center exchange integrals which are required to separate different terms within a configuration, (like singlets and triplets in $\pi^* \leftarrow n$ transitions). At any rate, the program allows one to choose between the CNDO and the INDO formulations, (and also between different *versions* of them, corresponding to different parametrizations). The method has been widely tested, yielding good results for aromatic molecules^{70,71}, aromatic molecules in a crystal field⁷², and, more specifically, for indole⁵⁹.

Zerner's program allows the selection of different types of electron repulsion parameters, like Mataga-Nishimoto⁷³, Ohno-Klopman⁷⁴, Pariser⁷⁵, and theoretical parameters. A general formula for the electron repulsion between two atoms A and B, at a distance R_{AB} is given by Callis *et al.*^{59,71}

$$\gamma_{AB} = \left(R_{AB}^n + [0.5(\gamma_A + \gamma_B)]^{-n} \right)^{-1/n} \quad (4)$$

In the Mataga-Nishimoto scheme, $n=1$ and R_{AB} is divided by 1.2⁶⁶. Meanwhile, $n=2$ in the Ohno-Klopman formulation. As pointed out by Callis⁵⁹, when n increases, the splitting between 1L_a and 1L_b decreases because γ_{AB} decreases more gradually with R_{AB} . Zerner's program also permits one to choose scaling factors for the interaction between molecular orbitals, (see METHODS section).

As stated in the previous section, CI can be used to include the electron correlation. The program allows for the inclusion of up to 210 configurations. Zerner *et al.*^{66,76,77} have shown that the use of singly excited configurations with Mataga-Nishimoto electron repulsion yields results in good agreement with experiments

for benzene and related molecules. This procedure was previously tested by Jaffé *et al.*⁷⁸ for CNDO. Callis⁵⁹, working on a suggestion by Zerner, has shown that this procedure is not the only one to effectively reproduce experimental results. He obtained good agreement by using doubly excited configurations along with Ohno-Klopman repulsion, because, although the doubly excited configurations bring the 1L_b state farther down than the 1L_a , the use of Ohno-Klopman repulsion, (and different interaction scaling factors), reduces the gap between the two states and compensates for the previous effect.

In our work, we chose to follow the first scheme, since it is easier to implement, especially when working with thousands of different geometries, as in our case. To that effect, (see METHODS section), we used a total of $14 \times 14 = 196$ excited configurations; that is, those formed from promotion of one electron from any of the 14 highest occupied to any of the 14 lowest unoccupied molecular orbitals.

Molecular Mechanics

Molecular Mechanics differs from molecular orbital methods in that no wavefunctions are sought. The idea behind molecular mechanics is that an appropriate potential energy function for the system, (where each atom is basically treated as a point charge), can be calculated and the geometry optimized towards the minimum of such potential energy.

Its expression is a rather complicated one, and can depend on a lot of parameters. For instance, a very simple expression for the potential energy for CO_2 could be as the one shown in Equation 5:

$$V = \frac{1}{2}k_{CO}(r_1 - r_{CO})^2 + \frac{1}{2}k_{CO}(r_2 - r_{CO})^2 + \frac{1}{2}k_{OCO}(\theta - \theta_{OCO})^2 \quad (5)$$

where k_{CO} and k_{OCO} are force constants, and r_{CO} and θ_{OCO} are values for the standard length and angle of such bond types. These magnitudes must be input into the program, (or the program has to store them). It must be pointed out that the expression for V in Equation 5 depends on r_i , (intermolecular distances), so it can be minimized.

This expression for V , however, is extremely simplified. As an example, the Central Valence ForceField, (CVFF)⁷⁹, includes up to eleven different terms, (Equation 5 includes only one term for bond distances and one for bond angles). The first four of those eleven are what is normally called *diagonal terms*. They include the energy of deformation of bond lengths, angles, torsional angles, and out of plane interactions. The other seven terms correspond to the *off-diagonal terms* and represent couplings of the diagonal deformations.

It is clear from Equation 5 that this method requires heavy parametrization, because each term needs at least two parameters for each type of bond included. CVFF has parameters for 50 different types of atoms and ions, thus, the number of bonds, angles, and dihedrals is going to be rather large.

Another widely used forcefield is the AMBER forcefield, due to Kollman *et al.*^{80,81}, which has six terms. The first three handle the deformations of bonds, angles, and dihedral angles, the fourth and fifth terms correspond to the van der Waals and electrostatic interactions, and the sixth corrects the electrostatic interaction for a hydrogen bond.

One important feature of molecular mechanics relies on the algorithm used in the minimization. There is a wide variety of algorithms, but we will mention here only the two that will be used in our calculations.

The *steepest descents* algorithm is the simplest of the methods. It just calculates the gradient along each coordinate and follows that path. After each step, the previous direction is substituted by the newly calculated, and the process is repeated until the convergence criterium has been met. The disadvantage of this method is that it is rather slow, especially when the system approaches a minimum. However, the advantage this method has is that it does converge even when far from a minimum. This is crucial in calculations involving many atoms, where the starting geometry is normally not very good. Other methods do not converge to a minimum in this case, but *steepest descents* does, even if the system does not behave harmonically.

The other method we used is the *Quasi-Newton-Raphson* algorithm, also known as the *variable metric method*, developed by Fletcher and Powell⁸². It is based on the *Newton-Raphson* method, which, besides using gradient information, uses the Hessian matrix calculated from second derivatives, (curvature), to predict where the function will change directions, (which indicates that it will pass through a minimum). The *Quasi-Newton-Raphson* method calculates the Hessian matrix numerically from the first derivatives, rather than analytically from second derivatives, and, therefore, it is much faster. This method is not as forgiving of bad initial structures as *steepest descents* is, thus, it is most suitable to be used in small systems where the initial geometry is not that far from a local minimum.

Molecular Dynamics

Molecular Dynamics can be considered a generalization of molecular mechanics in the sense that the system not only has a potential energy term but is also given kinetic energy and is allowed to change in time. The same potential energy term that was mentioned in the previous section is used, but the system does not evolve along the line that minimizes the gradients. Rather, the gradients of the potential energy along each coordinate correspond to the force applied to the particles according to Newton's law,

$$F_1 = -\frac{\partial V}{\partial r_1} = m_1 \frac{\partial^2 r_1}{\partial t^2} = m_1 a_1 \quad (6)$$

In trying to solve this equation, we face the same problem that was faced when trying to solve the Schrödinger equation, namely that there is no closed form solution for a system of three or more particles. Thus, a numerical solution has to be found, and the new position of each particle has to be approximated by a Taylor expansion,

$$r(t + \Delta t) = r(t) + \frac{\partial r}{\partial t} \Delta t + \frac{\partial^2 r}{\partial t^2} \frac{\Delta t^2}{2} + \frac{\partial^3 r}{\partial t^3} \frac{\Delta t^3}{6} + \dots \quad (7)$$

To calculate the new position, it is necessary to know the original position, velocity, and acceleration, and it is also required to know the value for higher order terms. As a result of the approximation made here, there arises a limitation on the time step used. The best results for systems like ours are obtained when $\Delta t \leq 1$ fs, because the integration algorithms require that velocities and accelerations are virtually constant over

the time step.

Another important issue here is that of temperature control. The temperature is initialized by assigning velocities to the ensemble of molecules, according to the Maxwell-Boltzmann equation,

$$f(\mathbf{v})d\mathbf{v} = \left(\frac{m}{2\pi kT}\right)^{3/2} \exp\left(-\frac{m\mathbf{v}^2}{2kT}\right) 4\pi \mathbf{v}^2 d\mathbf{v} \quad (8)$$

which expresses the probability for a molecule of mass m to have a velocity $\mathbf{v}$ at a temperature T , (k is the Boltzmann constant). When dynamics is started, (initialization period), the velocities are scaled at every step to instantaneously achieve the desired temperature. However, in later stages of the calculation, the scaling has to be much smoother, in order to not introduce artifacts that can affect the results. The best method to achieve the scaling has been developed by Berendsen and coworkers⁸³. Each calculated velocity is scaled by a factor λ , given by Equation 9.

$$\lambda = 1 + \frac{\Delta t}{2\tau} \left(\frac{T_0}{T} - 1 \right) \quad (9)$$

where T_0 is the desired temperature, T is the temperature corresponding to the distribution of velocities, and τ is the characteristic relaxation time, which can be adjusted to obtain better results.

A big drawback of both molecular mechanics and dynamics consists on the fact

that all the calculations are purely classical. Thus, there are some systems that cannot be accurately described, like electron or proton transfer, or bond breaking or formation, since they have to be treated by means of quantum mechanics methods.

Periodic Boundary Conditions

In trying to simulate a liquid solution, a common problem is that of finding an adequate geometry for the system. The most obvious solution would consist on surrounding the solute with a drop of solvent. However, this is not an accurate description, because it is actually equivalent to a *tiny* sphere of a few angstroms in diameter surrounded by vacuum. This is the reason why periodic boundary conditions, (PBC), are used. In this arrangement, the solute is surrounded, not by a sphere, but by a cube of solvent. This cube is reproduced by the program, and an "infinite" number of these reproductions, (*ghosts*), are stacked alongside the original in all directions of space. Therefore, the molecules in the original surface do not see a vacuum surrounding them. They actually see other solvent molecules, as they would in nature. This brings the problem of reducing the number of molecular interactions to a manageable size, which is taken care of by setting a cut-off distance where the interactions are no longer felt. Should a ghost molecule return inside the cut-off distance, its presence would be felt by the real molecule once again. When cut-off distances are chosen properly, MD runs even slightly faster using PBC than using a drop of solvent, (because some of the solvent-solvent interactions across the box are neglected while all of them are computed using a drop).

Another advantage of PBC is that the number of solvent molecules in the vicinity

of the solute is always the same. When using a drop, if the original geometry is strained, (especially for large systems), some of the solvent molecules can leave the system, acting as if they were evaporating.

Solvents

The Onsager-Mataga-Lippert Model

The Onsager reaction field⁸⁴ is the first useful formulation of the effect of a solute molecule in a medium of dielectric constant ϵ . The mathematical expression for the dielectric constant is attributed to Debye⁸⁵, who stated that

$$\frac{\epsilon - 1}{\epsilon + 2} = \frac{4\pi}{3} \sum N \left(\alpha + \frac{\mu^2}{3kT} \right) \quad (10)$$

where N is the concentration, (in molecules/volume), α and μ are the polarizability and permanent dipole of the molecule, k is the Boltzmann constant, T is the temperature, and the summation refers to the different components of the solvent mixture.

Onsager's model refers to a spherical solute of radius a inside a continuous medium of dielectric constant ϵ , (see Figure 3). In this model, the solvent polarizability can be expressed in terms of the solute size and the refractive index, (n), of the solvent

$$\alpha = \frac{n^2 - 1}{n^2 + 2} a^3 \quad (11)$$

The question of which molecular size to use is not a trivial one. Onsager himself

acknowledged that it is not reasonable to expect that the solute fills all the available space and that, therefore, a *void*, where $\epsilon=1$, should be added to the formulation. In our analyses, whenever a is needed in order to calculate some variable, we are going to calculate the molecular volume from density values, (D), in crystalline phase, assuming that the shape of the molecule is spherical, and using the molecular mass of the molecule, (M). From this size, a *radius* can be calculated, (see Equation 12). Alternatively, using the formulation described below, one can calculate values from spectroscopic results.

$$a = \sqrt[3]{\frac{3M}{4\pi D}} \quad (12)$$

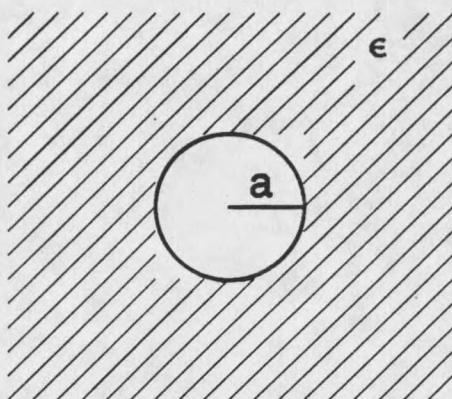


Figure 3. Onsager model: Spherical solute of radius a in a continuous solvent of dielectric constant ϵ

An additional problem in this model is the continuous nature of the solvent. As noted by Frisch *et al.*⁸⁶, this model, is not realistic when hydrogen bonds are present. Our calculations include hydrogen bonding between solvent molecules and, at least, partial hydrogen bond between solvent and solute. And, as it will be seen later, we can develop

a good quantitative analysis.

Further work by Ooshika⁸⁷, Mataga^{88,89}, Lippert^{90,91}, and McRae^{92,93} has improved Onsager's model and resulted in useful formulations for the spectral shifts reported in these previous references and in the work of Muiño and Callis⁵¹.

When an indole molecule goes from vacuum to solution in a polar solvent, both the ground and the 1L_a state are stabilized, although the magnitude of the stabilization is larger for the excited state as a result of the larger dipole moment of this state. The amount of stabilization induced on the ground state is given by Equation 13.

$$hc \Delta \bar{\nu}_{\text{ground}} = -\mu_g^2 \frac{2}{a^3} \left[\frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right] - \mu_g^2 \frac{2}{a^3} \left[\frac{n^2 - 1}{2n^2 + 1} \right] \quad (13)$$

where μ_g is the permanent dipole of the ground state of the solute, h is Planck's constant, and c is the speed of light in vacuum.

Although the expressions containing $f(n)$ in Equation 13 cancel out, they have been included to explicitly state the contribution of this term to the orientational polarizability of the solvent, (in the first bracket), and to its electronic polarizability, (second bracket).

Both components of the polarizability of the solvent play different roles in an excitation process. When the solute is promoted to the excited state, the solvent does not have time to reorient in the time elapsed in the excitation, (Born-Oppenheimer approximation). Thus, the orientational component of the polarizability remains constant during excitation. On the other hand, the electronic component remains in equilibrium with the solute dipole during the excitation process.

In calculating the stabilization of the excited state before the solvent has had time to respond, we expect the orientational polarizability contribution, (the first term on the right side of Equation 13), to be equivalent to that for the ground state, but substituting one of the ground state dipole moments in μ_g^2 for μ_e . To explain this partial substitution, one must consider that the solvent field is still equilibrated to the ground state, (thus a μ_g must remain in the expression), but the dipole of the state is now μ_e , (thus a μ_e must appear). Then, this contribution can be expressed as

$$-\mu_e \cdot \mu_g \frac{2}{a^3} \left[\frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right] \quad (14)$$

The contribution of the electronic polarizability of the solvent is not so obvious, because the dipole inducing solvent field is not the same in both states. We can calculate the difference in this contribution between ground and excited states by obtaining an expression for the work effected on the field by going from the ground to the excited state. If $\mu_\lambda = \mu_g + (\mu_e - \mu_g)\lambda$ and $d\mu_\lambda = (\mu_e - \mu_g)d\lambda$, then

$$-W = \int_0^1 \mu_\lambda \frac{2}{a^3} \left[\frac{n^2 - 1}{2n^2 + 1} \right] d\lambda = \left(\frac{\mu_e^2 - \mu_g^2}{2} \right) \frac{2}{a^3} \left[\frac{n^2 - 1}{2n^2 + 1} \right] \quad (15)$$

Then, the stabilization of the excited state is given by the sum of Equation 15 to the contribution of the ground state to the solvent electronic polarizability, (from Equation 13), plus the contribution of the orientational polarizability, (see Equation 14).

$$\begin{aligned}
hc\Delta\bar{\nu}_{\text{excited}} &= -\mu_e \cdot \mu_g \frac{2}{a^3} \left[\frac{\epsilon-1}{2\epsilon+1} - \frac{n^2-1}{2n^2+1} \right] + \\
&\quad - \left(\frac{\mu_e^2 - \mu_g^2}{2} \right) \frac{2}{a^3} \left[\frac{n^2-1}{2n^2+1} \right] - \mu_g^2 \frac{2}{a^3} \left[\frac{n^2-1}{2n^2+1} \right] = \\
&= -\mu_e \cdot \mu_g \frac{2}{a^3} \left[\frac{\epsilon-1}{2\epsilon+1} - \frac{n^2-1}{2n^2+1} \right] - \left(\frac{\mu_e^2 + \mu_g^2}{2} \right) \frac{2}{a^3} \left[\frac{n^2-1}{2n^2+1} \right]
\end{aligned} \tag{16}$$

By subtracting Equation 13 from Equation 16, we can calculate the absorption shift upon solution relative to vacuum^{51,94-96},

$$hc\Delta\bar{\nu}_a = -(\mu_e - \mu_g) \frac{2\mu_g}{a^3} \left[\frac{\epsilon-1}{2\epsilon+1} - \frac{n^2-1}{2n^2+1} \right] - \frac{\mu_e^2 - \mu_g^2}{2} \frac{2}{a^3} \left[\frac{n^2-1}{2n^2+1} \right] \tag{17}$$

Once the molecule is in the excited state, the solvent will start reacting to the new field induced by the solute. When the solvent finally is in *equilibrium* around the new charge distribution of the solvent, a similar analysis can be done, resulting in the formulation for the fluorescence shift relative to vacuum shown in Equation 18.

$$hc\Delta\bar{\nu}_f = -(\mu_e' - \mu_g') \frac{2\mu_e'}{a^3} \left[\frac{\epsilon-1}{2\epsilon+1} - \frac{n^2-1}{2n^2+1} \right] - \frac{\mu_e'^2 - \mu_g'^2}{2} \frac{2}{a^3} \left[\frac{n^2-1}{2n^2+1} \right] \tag{18}$$

Primes are used in this equation to indicate that these dipoles do not necessarily match those shown in Equation 16, because the polarization of the solvent field, (different

from the one induced when the solute was in the ground state), also induces changes in the dipole moments of the solute. The model we are using in our calculations does not include electronic polarizability of the solvent. If we subtract Equation 17 from Equation 18, and assume that $\mu_e'^2 - \mu_g'^2 \approx \mu_e^2 - \mu_g^2$, the electronic polarizability drops out^{97,98}

$$hc(\Delta\bar{\nu}_f - \Delta\bar{\nu}_a) = -(\mu_e - \mu_g)^2 \frac{2}{a^3} \left[\frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right] \quad (19)$$

which is convenient, since it allows us to predict this magnitude without having to worry about the lack of solvent electronic polarizability in the model. This expression can be thought as the difference in fluorescence shift between a fluid solution, ($\Delta\bar{\nu}_f$), where the solvent is allowed to relax after excitation, and a rigid solution, ($\Delta\bar{\nu}_a$), (for instance, indole in ethylene glycol+water at 80 K), where the solvent cannot react to the new dipole. This last scenario may not be completely accurate, because our calculations on 3MI in butanol at 0 K show that the solvent molecules can realign their dipoles according to the new solute dipole and cause a red shift of 1000-2000 cm^{-1} in a few picoseconds, (see RESULTS AND DISCUSSION).

If, instead of subtracting Equation 17 from Equation 18, we add them up together, (and divide by $2hc$), we get the following expression

$$\frac{\Delta\bar{\nu}_a + \Delta\bar{\nu}_f}{2} = -\frac{\mu_e^2 - \mu_g^2}{2hc} \frac{2}{a^3} \left[\frac{\epsilon - 1}{2\epsilon + 1} \right] \quad (20)$$

which is the quantity that Birks⁹⁹ calls the *shift in mirror symmetry wavenumber*. The importance of this expression stems from the fact that, from Marcus' work⁹⁴⁻⁹⁷, it can be shown that⁵¹

$$\Delta A_s = \frac{\Delta \bar{\nu}_a + \bar{\nu}_f}{2} \quad (21)$$

where ΔA_s is the difference in free energy of solvation between the excited and ground states.

Levy *et al.*¹⁰⁰ have compared their calculated ΔA_s to experimental $\Delta \bar{\nu}_a - \Delta \bar{\nu}_f$ values. On the other hand, Marcus⁹⁶ has shown that this expression is equivalent to two times the free energy to charge a molecule in the process of going from being uniformly neutral to having a charge distribution equal to $\pm$ the difference between the charge distributions of the ground and excited states.

From Equations 20 and 21, the sign of ΔA_s depends on the relative magnitudes of μ_e and μ_g , which is expected, because the free energy of solvation can decrease or increase after excitation, depending on whether the excited state dipole moment is larger or smaller than that of the ground state. On the other hand, if ΔA_s were proportional to $\Delta \bar{\nu}_a - \Delta \bar{\nu}_f$, (see Equation 19), one would get similar free energy changes regardless of which state has the larger dipole, which is counterintuitive. Callis⁵¹ has pointed out that the expression for ΔA_s corresponds to half the difference of the solute reaction field interaction potential energy. It is only half the difference because there is a quadratic relation between the solute-solvent interaction energy and the magnitude of the solute

charges due to the linear response of the solvent field.

Water Models

Important to any calculations in solution is the question of which theoretical model to choose for the solvent. Basically, two issues have to be addressed in picking it. First, the model must be fairly good in predicting the properties at hand. Second, it is important that it is as simple as possible in order to reduce computational time.

The search for a model for water has been and still is the subject of numerous studies. One of the first widely accepted was the ST2 model, due to Stillinger and Rahman^{101,102}. The early models tried to provide a method capable of mimicking solute-solvent interactions, and were parametrized to yield good results in some specific property.

Perhaps the most accurate model for non-rigid water is due to Watts¹⁰³. He fitted the model to spectroscopic data for the isolated water molecule and to the second virial coefficient of steam. He also corrected for long range interactions, with the result that his model was good in predicting the static lattice energy of ice. Unfortunately, this model needs 19 and 12 parameters for the intra- and intermolecular potential energies, respectively, making it computationally expensive for large systems.

In 1981, Berendsen *et al.*¹⁰⁴ proposed a new rigid model for water, called SPC, (single point charge). In this model, each atom of each water molecule is represented by a point charge, (O:-0.820 a.u., H:+0.410 a.u.), and each molecule presents a rigid geometry with $r_{OH}=1.0 \text{ \AA}$ and $\theta_{HOH}=109.47^\circ$. Key to this model is a Lennard-Jones potential that reproduces very well the solute-solvent distances.

Meanwhile, Jorgensen *et al.*^{105,106} developed the "TIP" series: TIPS2, TIP3, and TIP4P. These models are all flexible, and, therefore, they include some polarizability of the solvent. The TIPS2 and TIP4P models also include some electronic polarizability, because, although the positive charges, ($\sim +0.5$ a.u.), are centered in the hydrogens, the negative charge, (~ -1.0 a.u.), is not centered on the oxygen, but on a point $0.15 \text{ \AA}$ away, whose distance to the oxygen may change. The TIP3 model does not have this feature, but it is simpler, (faster), and more used. As SPC, it has three point charges, (O:-0.834 a.u., H:+0.417 a.u.), with $r_{\text{OH}}=0.957 \text{ \AA}$ and $\theta_{\text{HOH}}=104.52^\circ$, and it is also a good model to predict intermolecular distances.

The model used in these calculations, which is the default for Discover, (the molecular dynamics program we use), is labelled SPC, but is not a pure SPC model. Rather, it is a SPC/TIP3 hybrid, built according to the following specifications.

1. SPC Lennard-Jones potentials for oxygen.
2. SPC charges.
3. TIP3-like bond length and angles, ($r_{\text{OH}}=0.96 \text{ \AA}$ and $\theta_{\text{HOH}}=104^\circ$).
4. It is flexible.
5. Lennard-Jones parameters for hydrogen, ($A=10^{-8} \text{ kcal\AA}^{12}/\text{mol}$).

A regular SPC model does not need a Lennard-Jones parameter for hydrogen because the model is rigid. However, when the model is flexible, if the parameter is not included, it would result in the so called *Coulomb catastrophe*¹⁰⁷; that is, the hydrogens would be pulled away from their oxygens by the neighboring oxygen atoms.

Lau *et al.*¹⁰⁸ have found that the first peaks of the O-O, O-H, and H-H and the

second peaks of the O-H and H-H calculated radial distribution functions, (rdf), are within 0.1 Å of experimental values. The second peak of the O-O rdf has an error of 1.0 Å. The equilibrium density is within 3% of experimental value and the intermolecular energy has a 2% error, (~0.2 kcal/mol). On the other hand, the diffusion coefficient is off by a factor of 2. These results have been confirmed by Thompson¹⁰⁹, except for the second peak of the O-O rdf, which he does not observe.

Experimental

Early studies of indole^{1,4} were done by measurement of the absorption spectrum of the vapor phase or liquid solution of indole in a spectrometer. Thus, the molecule was excited with a non-coherent, low intensity source, and the linewidth of the exciting light was fairly broad. The use of lasers as an excitation source was a significant improvement, because the linewidth of the light is much narrower and allows for site selection¹¹⁰, which means that we can selectively excite different conformers of the same molecule or molecules which are in a particular type of environment.

In our experiments, we use the technique of laser induced fluorescence excitation, widely used to study indoles and similar molecules in all types of environments^{32,33}. In these experiments, the sample absorbs part of the light in the laser beam, but, instead of measuring the amount of light absorbed, what is measured is the fluorescence emitted by the excited molecules. In condensed phase, this measurement is generally equivalent to an absorption measurement of the 1L_a and 1L_b states, because, although the amount of collected fluorescence depends not only on the absorption, but also on the quantum yield

of the transition¹¹⁰, the quantum yield does not change with the excitation frequency. This behavior, however, is not characteristic of the gas phase. The expression for the quantum yield, (Φ_F), is:

$$\Phi_F = \frac{k_f}{k_f + k_{NR}} \quad (22)$$

where k_f is the rate constant for the fluorescence process and k_{NR} is the rate constant for non-radiative processes starting at the same level as the fluorescence. Obviously, if a very strong absorption is followed by total intersystem crossing, no signal would be collected using this method. In practice, for our work on indole in a supersonic jet, it has been found that at $\sim 1500 \text{ cm}^{-1}$ above the 1L_b origin the quantum yield decreases significantly and fewer transitions are characterized^{35,111,112}.

Supersonic Jet

The combination of laser induced fluorescence with expansion in a supersonic jet started in the 1970s. Levy *et al.*¹¹³ reported a fluorescence excitation spectrum of NO_2 that was much simpler than any other measured at room temperature. This is the main advantage of a supersonic expansion: it allows the "cooling" of the molecules, resulting in a significant decrease in the population of the vibrationally and rotationally excited states of the electronic ground state.

A supersonic jet, (see Figure 4), consists of a pressurized chamber at 3-10 atm, (a), with the sample and a carrier gas, (normally helium, but other noble or even non-noble gases can be used). This chamber is connected to another one, (b), at higher vacuum,

($>10^{-5}$ torr). In between these two chambers there is a small opening, or nozzle, whose diameter is in the order of 0.05 to 1 mm. When the molecules escape from the first to the second chamber, they acquire a large kinetic translational energy in the direction of the expansion. However, this energy is taken from the rotational, vibrational, and the other translational degrees of freedom, resulting in very low rotational, ($\sim 1-5$ K in our system), and low vibrational temperatures, as well as a smaller transversal Doppler effect¹¹⁰.

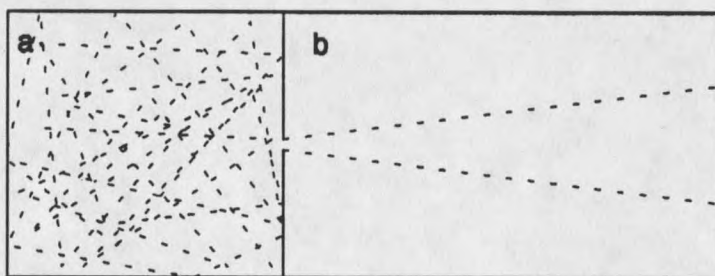


Figure 4. Schematics of a supersonic jet

As a result, most transitions originate at the $v=0$ state of the electronic ground state and each of the few transitions present in the spectra can be assigned to a normal mode, overtone, or combination band of the electronically excited state.

Two-Photon Spectroscopy

In the previous pages we described the technique of laser induced fluorescence. In general, the simplest way to reach the excited state consists of using a photon whose energy matches the gap between initial and final states. This is the basis of what is called one-photon absorption, or OPA, (see Figure 5). However, there is another way to reach

the final state: if, simultaneously, two photons arrive at the molecule, and the sum of their energies matches the energy difference between states, the photons can be absorbed and the molecule ends up in the excited state, even though each individual photon is not resonant with any individual state of the molecule. The two photons do not need to have the same energy, although they will in our experiments.

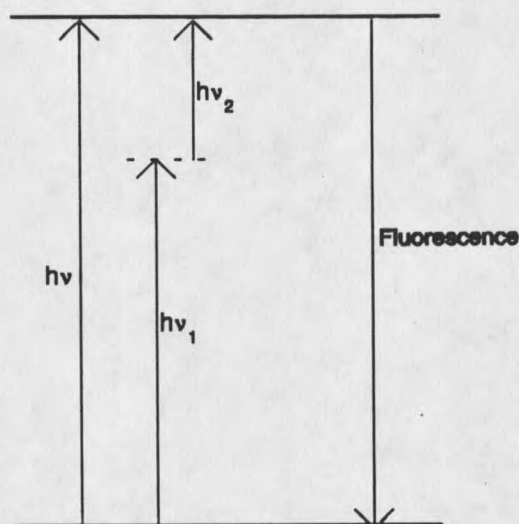


Figure 5. One- and two-photon absorption.

The first formulation about two-photon process was due to Göppert-Mayer¹¹⁴ in 1931. At the time her work was published, however, there was no light source intense enough to induce these phenomena. The two-photon formulation can be derived from perturbation theory. When the photon flux of the light source is high enough, second order perturbations are no longer negligible.

Two-photon spectroscopy is just a branch of a more general field dealing with multi-photon processes. An excellent theoretical treatment can be found in the work of

McClain¹¹⁵⁻¹¹⁷ and Schlag *et al.*¹¹⁸. In our experiments, we will be dealing with the particular case where the two photons are absorbed simultaneously, and the molecule has enough time to become spatially randomized before emission. Thus, no information about the emitted photon polarization is required or gained. Furthermore, as the absorption of the two photons takes place *simultaneously*, the molecule cannot move in that interval, and the photoselection refers only to the ground state and the final state reached by the two-photon absorption. Molson and McClain¹¹⁹ pointed out that even if the excited state undergoes internal conversion and then fluoresces, the photoselection information about the original excited state is conserved. This last statement is of paramount importance to our experiments because, independently of which one is the fluorescing state, we can get information about the state that was reached upon absorption. The rate of simultaneous absorption of two identical, non-resonant photons is^{117,120}

$$\text{rate} \propto |\mu \cdot S \cdot \mu|^2 I^2 \quad (23)$$

where μ and I are the unit polarization vector and the flux of the laser light, and S is the two photon tensor.

An expression for a component, (S_{ab}) , of a 3×3 two photon tensor is also widely available in the literature^{117,120,121}

$$S_{ab} = \sum_i \frac{a_i \cdot b_i}{v_{ig} - v_{laser}} \quad (24)$$

where a and b are x , y , and z , and the summation is extended to all energy levels,

including ground, (g), and final, (f), states.

Inspection of Equation 23 reveals that there is a condition required in order to have a strong two photon absorption: The laser intensity needs to be strong. In particular, for our system, where the laser light comes from a pulsed nanosecond dye laser, the laser beam needs to be tightly focused in order to supply enough photon flux, (see METHODS section).

Polarization

It was stated previously that, through photoselection, we can get information about the nature of the excited state. From McClain's¹¹⁵ work, Equation 25 gives the expression for the fluorescence intensity when the two absorbed photons are identical and the emission is isotropic.

$$I = \sum_{i,j=1}^4 P_i M_{ij} Q_j \quad (25)$$

where P_i are geometric factors depending on the polarization of the absorbed and emitted photons, M is a matrix that binds P_i and Q_j , and Q_j is a factor which depends on the two-photon tensor, (see Equation 23), and the fluorescence transition dipole.

The key factor here is P_i . As P_i depends on the polarization of the light, it is expected that the fluorescence intensity will change if we change the polarization. To this effect, McClain¹¹⁶ has defined several molecular parameters. Two of them are presented in Equations 26 and 27, (see also Callis¹²⁰).

$$\delta_F = \sum_{a,b} S_{aa} S_{bb}^* \quad (26)$$

$$\delta_G = \sum_{a,b} S_{ab} S_{ab}^* \quad (27)$$

From these parameters, a third one, Ω , can be defined. This Ω is the so called *two-photon polarization* or *polarization ratio*, which is the ratio of fluorescence intensities obtained when using circularly and linearly polarized light.

$$\Omega = \frac{I_{\text{CIRCULAR}}}{I_{\text{LINEAR}}} = \frac{3 - \delta_F/\delta_G}{2 + \delta_F/\delta_G} \quad (28)$$

(we are still under the assumption that the molecules are randomized before emission).

Under these conditions, it can be shown that Ω can vary only between 0 and 1.5¹²⁰.

Experimental results^{32,33,35} have consistently shown that Ω is approximately 1.5 for the

1L_b state and 0.5 for the 1L_a state. These measurements agree quite well with calculated

INDO/S values¹²⁰. Transition to the 1L_b state is favored when the polarization of the

second photon is perpendicular to that of the first one. This scenario is enhanced by the

use of circularly polarized light. On the other hand, absorption to the 1L_a state is favored

when the polarizations of both photon are parallel, and this is obviously enhanced by the

use of linearly polarized light. Thus, two photon spectroscopy using polarized light

provides a means of distinguishing 1L_a from 1L_b transitions.

METHODS

Theoretical Methods

Hardware

With the exception of one set of simulations, which will be described later, all the calculations reported here have been performed on a Silicon Graphics 4D25TG Personal Iris, with an IP6 Processor operating at 20 MHz and a MIPS R2000A/R3000 processor chip.

Software

INDO/S

INDO/S was obtained from Professor Michael Zerner^{66,67} and adapted by Patrik Callis to run in the UNIX environment. A configuration interaction of 196 singly excited states, (14×14), was used to calculate the transition energies for the 1L_a and 1L_b states. Mataga-Nishimoto gamma integrals⁷³ were used because they have been shown by Callis to better reproduce experimental results for indoles when used with singly excited configurations.⁵⁹ Also, from this work of Callis' we chose the corresponding scaling factors for the molecular orbital interactions: 1.000 for σ_s - σ_p , 1.267 for σ_p - σ_p , and 0.585 for π - π interactions. The effect of the solvent on the solute was treated as purely coulombic, and thus, it was added to the diagonal elements of the Fock matrix. This also has the advantage of not introducing the molecular orbitals of the solvent molecules into

the calculation, greatly reducing the computational time required for these calculations. The atomic charges for the water molecules were picked from the SPC model¹⁰⁴: -0.820 a.u. for oxygen and +0.410 a.u. for hydrogen. For the other solvents, an optimized geometry was obtained using MOPAC6, (adapted by V. Danilov), followed by an INDO/S calculation performed on this geometry to obtain the charges from the Löwdin basis atom densities. A list of the charges for the three solvents is given in Table 1.

Table 1. Atomic charges for three of the solvents used.

BUTANOL		METHANOL		DME	
Atom	Charge	Atom	Charge	Atom	Charge
C1	0.0970	C	0.1100	C1	0.1060
H11	0.0520	H1	0.0210	H11	0.0220
H12	0.0460	H2	0.0210	H12	0.0220
O1	-0.5070	H3	0.0210	H13	0.0220
HO	0.2830	O	-0.3910	O	-0.3440
C2	-0.0570	HO	0.2180	C2	0.1060
H21	0.0320			H21	0.0220
H22	0.0450			H22	0.0220
C3	-0.0610			H23	0.0220
H31	0.0450				
H32	0.0350				
C4	-0.1140				
H41	0.0310				
H42	0.0350				
H43	0.0380				

Molecular Dynamics

Molecular Dynamics was run using the program Discover v. 2.80, from Biosym. The input files were prepared using the graphics interface InsightII. A single 3MI molecule, (or indole in some simulations), was placed in the center of a periodic boundary conditions cubic box. The number of solvent molecules added to the box resulted in a total density of $\sim 1 \text{ g/cm}^3$ for water and $\sim 0.8 \text{ g/cm}^3$ for the other solvents. In one set of simulations involving 3MI in water and another one, involving 3MI in butanol at 0 K, the solute was placed at the center of a spherical drop of solvent.

The geometry for the solute was obtained from a tryptophan crystal structure¹²² and adapted for planarity of the molecule, (see Figure 6). Geometries for the 1L_a and 1L_b states were estimated using INDO/S. To obtain them, the bond length variations with respect to the ground state geometries were calculated by multiplying the corresponding off-diagonal terms in the π -bond order difference matrices by the scaling factor -0.17^{59} , (see Figure 7), or $-0.22^{33,123}$. The charges for the solute molecule were also obtained using INDO/S. In some instances, the charges calculated for the isolated molecule were used, but, most frequently, the charges were calculated for the molecule surrounded by the solvent cloud. Two different approaches were tried. First, a model we called *semipolarizable* was developed. Charges were calculated for 1000 different configurations and averaged, for ground and excited states. The charges calculated in this way were kept constant during the whole molecular dynamics simulation. Later, a second model, called *polarizable*, was introduced. In this model, after a small number of molecular dynamics steps, the coordinates of the whole system were extracted and used as input for INDO/S,

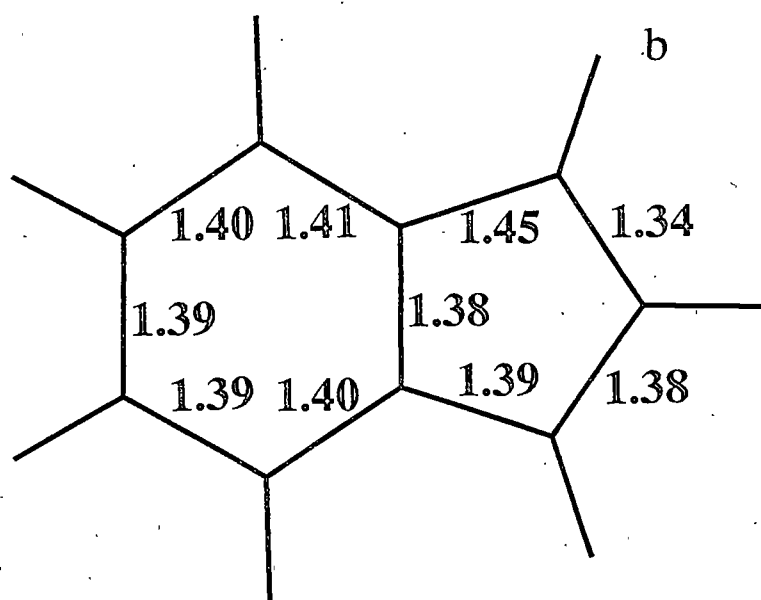
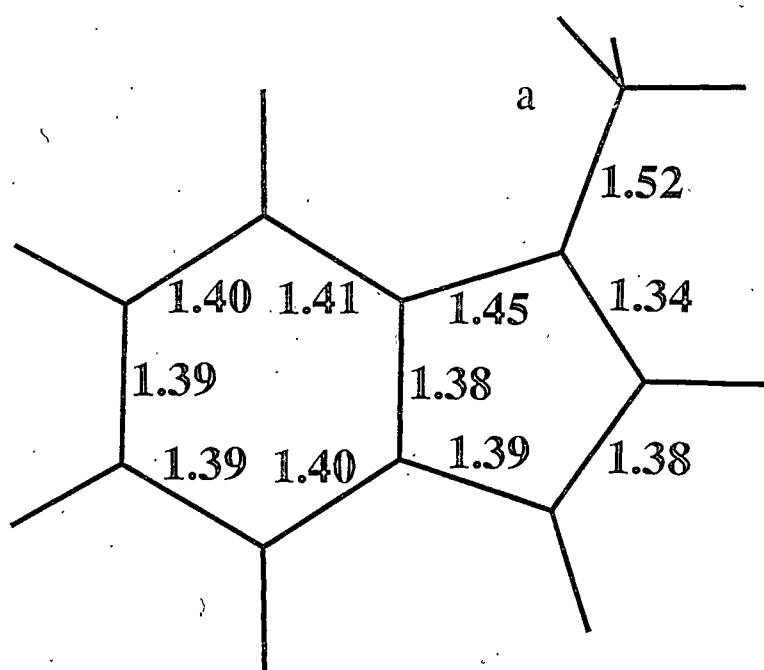


Figure 6. 3MI, (a), and indole, (b), geometries in the ground state. The C-H and N-H bond distances are 1.08 Å.

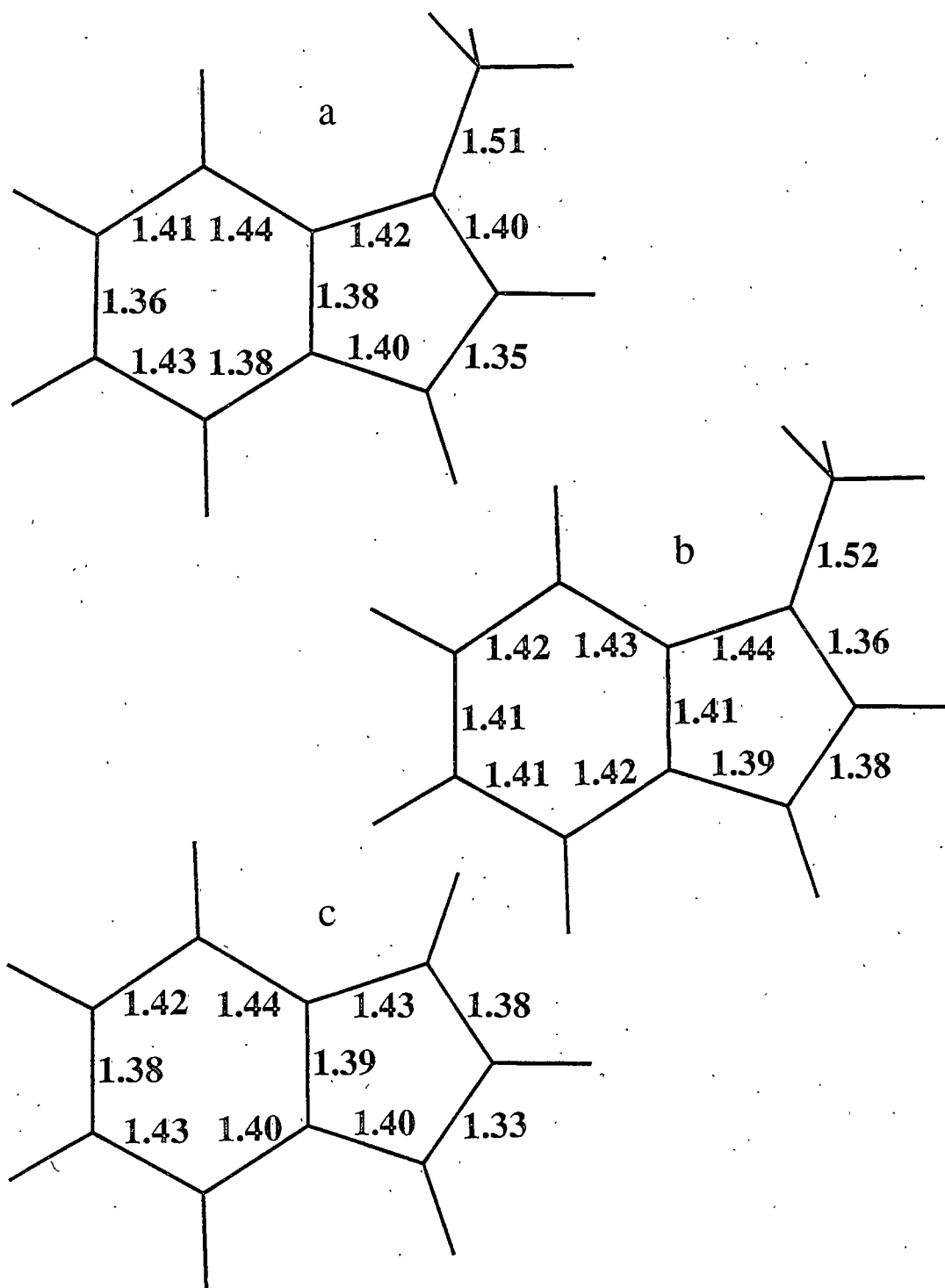


Figure 7. Geometries for the 1L_a , (a), and 1L_b , (b), states of 3MI, and the 1L_a state of indole, (c). The C-H and N-H bond distances are 1.08 Å

which calculated a new set of charges for the solute that was used for the next dynamics steps. The same solvent charges used in INDO/S were used here. Independently of the model used, output from any of the programs, (INDO/S or Discover), was going to be used as input for the other one. To adapt the files to the required format, we wrote our own code, (see Appendix A for a list of programs and files), using Fortran77 on the Silicon Graphics.

We chose to use the CVFF forcefield⁷⁹, which is the default in Discover. As stated before, one set of simulations was performed without using Discover. The program CHARMM¹²⁴ was run for these particular simulations on the CRAY YMP at the Pittsburgh Supercomputing Center. This program has its own forcefield, which includes the TIP3 model^{105,106} for the water molecules. No significant differences were found with respect to Discover, thus, CHARMM was no longer used.

It was necessary to introduce cutoff distances when using periodic boundary conditions, in order to reduce the number of non-bonding interactions so the calculations could be performed in a reasonable amount of time. The cutoff distance was set at 10 Å for the Discover calculations and 9 Å for those run in CHARMM.

In the CVFF forcefield, there are three switches that can be used to specify whether particular additions or modifications are to be added to the potential energy: The first switch allows the inclusion of charge-charge interactions (coulombic); the second determines whether or not cross terms (or coupling of the deformation of internal coordinates) are to be included; finally, the third switch allows the addition of a Morse function to the potential energy terms so that they are no longer harmonic. In our

calculations, the first two options were used. Some experimentation was done on the use of Morse potentials, but it was found that, due to a bug in the program, the calculations crashed when these potentials were included along with solvent molecules.

Molecular Mechanics

Discover was also used to run molecular mechanics under the same conditions, (forcefield, cutoffs,...) that were used in molecular dynamics. The *steepest descents* algorithm was chosen to find a minimum for the system because it is the most convenient to use in the presence of many atoms. The criterion for a conformation to be considered a minimum, (in the presence of solvent), was established at the largest gradient being less than 0.5 kcal/Å.

Some calculations were performed where the number of atoms was small, for instance, when searching for minima for an indole-solvent complex or for rotational conformers of tryptophan. In these cases, the *Quasi-Newton-Raphson* algorithm⁸² was chosen because it converges faster for small systems. Morse potentials were included and the criterion for a minimum was set for the largest gradient being less than 0.0001 kcal/Å.

Procedure

Solution

Once the solute was surrounded by the solvent cloud and the parameters for the calculation were set, as previously described, a molecular mechanics minimization was done to eliminate all possible strains in the system that would not allow molecular dynamics to run. At all times during this minimization as well as during dynamics, the

solute geometry was kept fixed, in order to not let molecular vibrations interfere with the solvent effects, which was the object of our study.

After a local minimum was found, (according to the criterion described in the previous section), molecular dynamics was initialized for 1000 steps, (1 fs each), in order to bring the temperature of the system to 300 K by scaling of the velocities of the solvent molecules. Then, the ensemble was equilibrated and homogenized for another 99 ps. Such a long equilibration is necessary to bring the system to a Boltzmann distribution of energies, as evidenced by the small oscillations in the total energy, (at earlier stages, the oscillations are large, indicating the scaling of the kinetic energy is frequently under way). After these elapsed 100 ps, the system was ready to start our calculations, as indicated by the smaller fluctuations in the energy of the system.

Absorption

The solute was always kept in the ground state to calculate the absorption redshift. The calculations started with the equilibrated system at a time arbitrarily called 0. Molecular dynamics was run for 5 fs and the coordinates for the ensemble were obtained and input in INDO/S which then calculated the transition energies, permanent dipoles, and oscillator strengths for the 1L_a and 1L_b states and the ground state charges, (Appendix A contains a list of typical command and data files). The charges were then changed in the molecular dynamics input, so this program could use the new charges for the next 5 fs. This procedure was repeated 6000 times, so a total of 30 ps of dynamics was simulated. We had previously performed shorter simulations using 1 fs dynamics runs between INDO/S calculations, and we had found that 5 fs intervals between transition energy

calculations capture all the structure in the solvent induced fluctuations.

After the calculation was finished, a histogram of the frequency of the transition energies was generated using Quattro Pro. The chosen transition energy intervals were 50 cm^{-1} for 1L_b and 200 cm^{-1} for 1L_a . The maxima of these two histograms were compared to the transition energies for both states in vacuum and thus the Stokes shift was calculated for each of the solvents.

Mathematical manipulation of the solvent induced transition energy fluctuations can yield information about time resolved fluorescence redshift. If we calculate the autocorrelation function, (see Equation 29), of these fluctuations, this function should be equivalent to the calculated direct response^{45,46,125} (see next section), in the event that the solvent is not significantly polarized by the solute⁴⁸

$$C_{eg}(t) = \frac{\langle \Delta v_{eg}(0) \Delta v_{eg}(t) \rangle}{\langle \Delta v_{eg}(0)^2 \rangle} = \frac{v(t) - v(\infty)}{v(0) - v(\infty)} \quad (29)$$

Fluorescence

In order to calculate the fluorescence redshift, the solute was excited to the 1L_a state at some point in the calculation. This was achieved by instantaneously changing the geometry and charges of the molecule from ground to 1L_a state values.

After an equilibrated ensemble was generated, the calculation was ready to start. The procedure varied depending on whether the solvent was *semipolarizable* or *polarizable*. The time scales were different for water than for the other solvents. The values shown in the following description correspond to water, while the values in

parentheses are the ones used with the other solvents.

When the solvent was *semipolarizable*, (as described in the Molecular Dynamics section), molecular dynamics ran with the solute in the ground state for 0.5 ps (1.25 ps for the non-aqueous solvents) and the coordinates for the system were read and stored every 10 fs (25 fs). Then, the geometry and charges of the solute were changed to the 1L_a values, and molecular dynamics ran for another 4 ps (10 ps). During the first 200 fs (400 fs) after excitation, the coordinates of the ensemble were read and stored every 5 fs (10 fs). For the rest of the run, these coordinates were extracted every 10 fs (25 fs). The 50 structures read for the ground state and the 420 read for the excited state were then input into INDO/S, which generated transition energies, dipoles and oscillator strengths for both excited states that would be plotted as a function of time. Normally, ten calculations, (each with a different starting point), were performed under a particular set of conditions and averaged.

When the solute was *polarizable*, the time scales involved were similar, but molecular dynamics did not run continuously. Instead, every time the ensemble coordinates were extracted, molecular dynamics stopped and an INDO/S calculation followed. Along with the transition energies, dipoles, and oscillator strengths for the excited states, new charges for the solute were calculated and input in the molecular dynamics calculations. As it was done for the *semipolarizable* model, a set of ten runs was recorded and averaged together.

Finally, some calculations were done in butanol at 0 K. The procedure was different from those described before. The system was equilibrated at 300 K. Then, 25

starting configurations were generated, each of them at 1 ps from the previous one, (still at 300 K). Every configuration was cooled down for 8 ps to within 0.05 K of 0 K. Molecular dynamics was run for 2 ps, still in the ground state, and coordinates were recorded every 25 fs. Then, the solute was excited to the 1L_a state and molecular dynamics ran for another 5 ps. During the first picosecond, coordinates were recorded every 10 fs. For the rest of the calculation, coordinates were recorded every 25 fs. Once again, they were fed into INDO/S to get spectroscopic information on the excited states. The 25 calculations were averaged, but individual analyses on some of them were also done. For instance, the fluorescence decay in some runs was Fourier transformed to get information on the frequencies of the decay.

Rotational Conformers of Tryptophan in Vacuum

Molecular mechanics, (using Discover), was used to study the number and relative population of the rotational conformers of non-zwitterionic tryptophan. The geometry of the indole ring was kept fixed while each of the angles in the side chain was modified in steps of 60° . Then, molecular mechanics was run until a local minimum was reached. More than 60 different minima were found. Their relative population was calculated by means of the Boltzmann factor for the energy of the minimum. Also, an INDO/S calculation was performed on each minimum to determine the 1L_a and 1L_b transition energies.

Experimental methods

Chemicals

Indole from Aldrich (99+%), lot number 03231AZ, was used without further purification. Bulk deionized water from our lab faucets was pure enough for these experiments. D₂O (99.9%) was purchased from CIL and methanol (HPLC grade) was obtained from Fisher. Ultra high purity helium (99.999%) was necessary for these experiments. Lower grade helium was tested, but it contained enough impurities to produce a significant number of complexes with indole that showed in the spectra. Helium was obtained from General Distributing.

The dyes for the laser, (C540A, R590, and R610), were obtained from Exciton. Methanol, already described, was used as solvent for the dyes.

Instrumentation

Our experimental setup can be seen in Figure 8.

A Q-switched Lumonics Nd:Yag (HY-200) was used to pump a Lumonics HD300 dye laser¹²⁶⁻¹²⁸. Either the second (532 nm) or the third (355 nm) harmonics were used to pump the dye laser. The lasers operated at 20 Hz and were driven by an Iota One controller, (from General Valve), which also was used to run the nozzle, (also from General Valve), in the jet, (Appendix B contains information about the connections and parameters of operation).

The supersonic jet was built by Jungst Scientific according to the specifications provided by Callis and Sammeth. It consists of a cubic stainless steel hollow box of 25

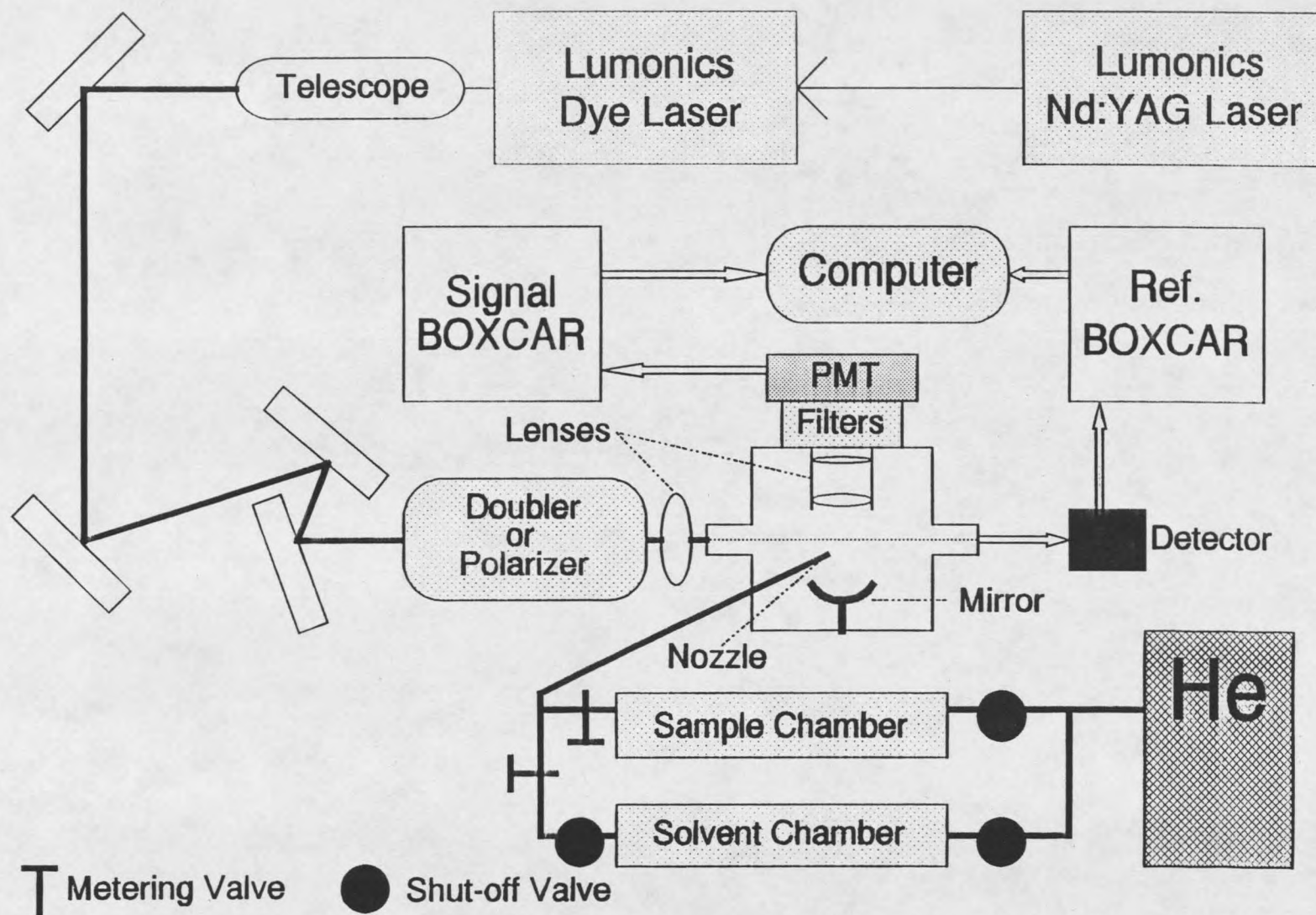


Figure 8. Experimental setup.

cm per side. Two cylindrical tubes ($\phi_1=2.5$ cm) guided the laser light in and out of the jet along its X axis. Each of them has three internal baffles ($\phi_1\sim 4$ mm) to prevent outside light from entering the jet. Two quartz windows sealed with Apiezon were placed at the outer ends of the tubes and perpendicular to the laser beam. The inside ends do not touch each other. Rather, they are approximately 15 cm apart.

A heated sample chamber was located above the jet and was connected to the nozzle directly along the Z axis. A second chamber, containing the solvent could be connected to the carrier gas line, (see Figure 8). The amount of solvent in the system was regulated by metering valves. The nozzle could be displaced along the Z axis. For the one-photon experiments, the nozzle was 2.0 cm above the center of the chamber, (which was crossed by the laser beam). For the two-photon experiments, the nozzle was 0.5 cm above the center of the chamber.

To aid in the fluorescence collection, a series of optical instruments was aligned along the Y axis. A coated spherical mirror, ($\phi=60.0$ mm, $f.l.=25.0$ mm), from Melles-Griot, was placed opposite the PMT, (described later), on the other side of the molecular beam. Fluorescence was focused on the PMT by means of two S1-UV grade fused silica lenses, from Esco, ($\phi=50.8$ mm, $f.l._1=50.8$ mm, and $f.l._2=101.6$ mm).

A vacuum was constantly kept in the chamber by means of a series of pumps. A Varian 15.8 CFM roughing pump, backed a Varian VHS-6 diffusion pump, with a speed of 3000 l/s (He). We placed a gate valve and a cryopump between chamber and diffusion pump. The Edwards GVA 060M gate valve allowed isolation of the chamber from the pumps, so they could be running while modifications were made inside the chamber. The cryopump, which was not cooled, simply acted as a trap to prevent oil spillage from the

diffusion pump into the chamber. The diffusion pump could be bypassed, so it was not necessary to stop it when the chamber needed to be roughed. Two Varian stainless steel thermocouple gauges (model 531) connected to Varian 801 controllers measured the chamber and backing pressures, while a more precise Varian 524-2F cold cathode ionization gauge connected to a Varian 860A cathode gauge control recorded the pressure inside the chamber when this was below 10^{-3} torr.

As mentioned before, the Iota One controller drove both the nozzle and the lasers. However, due to the mechanical nature of the nozzle, as opposed to the electronic nature of the laser firing, the effective opening of the nozzle took place after the laser beam had already passed through the jet. Therefore, it was necessary to introduce a delay in the laser trigger to avoid this problem. First, we used a homemade electronic delay with a range from 0.4 to 2.5 ms. Unfortunately, this delay was very sensitive to ground current loops and the circuit breaker in the Nd:YAG laser was frequently thrown. Thus, we adapted a EG&G Princeton Applied Research model 162 boxcar averager to act as a delay. Finally, we acquired a Stanford Research Systems DG535 Digital Delay/Pulse Generator, (see Appendix B for details). All three delays performed equally well and the results were similar.

When doing one-photon spectroscopy, the green laser light emitted by the dye laser was doubled into UV light by means of an Inrad Autotracker II frequency doubler. To generate circular and linear polarizations for the two-photon spectra, a double Fresnel rhomb half-wave plate followed by a Fresnel rhomb was used¹²⁹. Also in two-photon spectroscopy, a UV grade fused silica focusing lens, from Esco, ($\phi=50.8$ mm, $f.l.=700$ mm), was placed at the jet entrance, at approximately 460 mm away from the center of

the jet chamber, in order to focus the laser beam at 240 mm beyond that point. This was the distance that yielded the best experimental results by increasing the laser power without significantly reducing the excitation volume.

Fluorescence was collected at right angles with a Hamamatsu R928 PMT cooled at -25°C . The output was amplified and gated using Evans Associates circuitry included in a boxcar triggered by a pair of photodiodes placed at the dye laser exit. This boxcar was built by Anderson and Callis¹²⁸. A diaphragm with a 5 mm opening and a WG-320 filter (2 mm thick) were used to prevent scattered light from reaching the PMT during one-photon spectroscopy. The transmittance of a 1 mm WG-320 filter is less than 10^{-5} for wavelengths between 270 and 290 nm. Transmittance goes up very quickly, reaching 0.8 at 325 nm. A 3 mm thick UG-11 filter, a saturated aqueous solution of NiSO_4 , and a diaphragm with a 1 cm opening were used in two-photon experiments for the same purpose. A UG-11 filter transmits very well in the region of 325 nm, where the fluorescence is collected, but its transmittance, (per mm), at 560 nm is also less than 10^{-5} . NiSO_4 was used to block the Raman scattered light produced by the quartz windows when the laser beam passed through them. A saturated solution of NiSO_4 does not transmit from 680 to 750 nm, the region of the spectrum corresponding to the scattered light, but does give a window of transmission around 325 nm. The laser intensity was measured by a homemade quantum counter containing Rhodamine B built by Jones and Callis¹³⁰.

Data were recorded in a PDP-11 computer through a Data Translation A/D board. A program to control the laser scan and to drive the polarizer was written by Anderson, Callis, Jones, Rehms, Theiste, and Williams, and later modified by Sammeth and Muiño.

Parameters and Operation

The pressure in the sample chamber was set at approximately 4 atm, while the temperature was 44°C in the one-photon experiments and 90°C in the two-photon experiments. When the nozzle was not in operation, the pressure in the jet chamber was kept at $\sim 5 \times 10^{-6}$ torr. The pressure raised to $\sim 5 \times 10^{-5}$ torr when the jet was firing. The Iota One controller triggered the nozzle at a 20 Hz rate with an opening of 600 μ s per shot. The laser was fired with a delay of ~ 500 μ s with respect to the nozzle trigger.

The second harmonic of the Nd:YAG laser was used to pump the dye laser when using R590 (53 mg/l) or R590+R610 (53 mg/l and 7 mg/l). The third harmonic was used to pump the dye laser when using C540A (1080 mg/l). The laser beam was expanded at the exit of the dye laser using an inverted galilean telescope, in order to bring its diameter to ~ 4 mm, which is the minimum required to prevent burning of the optical coatings in the mirrors. These mirrors are used to align the laser beam through the jet. To aid in this procedure, the last two mirrors are placed as close as possible to the jet entrance, (see Figure 8). It is necessary, however, to leave enough room to place the frequency doubler or the polarizer and the focussing lens between the last mirror and the jet. The last mirror should not be mounted directly on the table. It should be bolted to an XY mount in order to assist in the fine adjusting of its position

The collected fluorescence and the laser signal were gated and integrated for 200 μ s in the boxcar, converted to digital output and directed to the computer, where they were stored. To normalize the signal, this was divided by the laser intensity in one-photon experiments and by the laser intensity squared in two-photon experiments.

RESULTS AND DISCUSSION

This section is divided into the same two categories as the METHODS section was. The first is a description of the theoretical work and a discussion of the results obtained for indole and 3MI in bulk solvents. The second refers to the experimental work on indole and van der Waals complexes, including as well a discussion of this work.

Theoretical Work

Figure 9 shows the 1L_a and 1L_b transition energy fluctuations for 3MI in the ground state in water at 300 K. At $t=0$, the molecule absorbed a photon and was excited to the 1L_a state. This excitation was simulated by instantaneously changing the charges of the solute from ground state to 1L_a values. It can be interpreted from the graph that this had the effect of reorganizing the solvent which, in turn, stabilized both excited states resulting in a red shift in the transition energies. At $t=2.0$ ps, the molecule emitted a photon and returned to the ground state, (simulated by a new change in the charges). This brought the transition energies to pre-excitation values.

Although this calculation was useful in establishing that this method could be used to study the transition energy fluctuations and their response to excitation, it is clear that several improvements were needed in order for it to yield good quantitative results. First of all, the question of the time step arose, that is, the question of how often it was necessary to calculate the transition energy fluctuations. To answer this, a calculation was

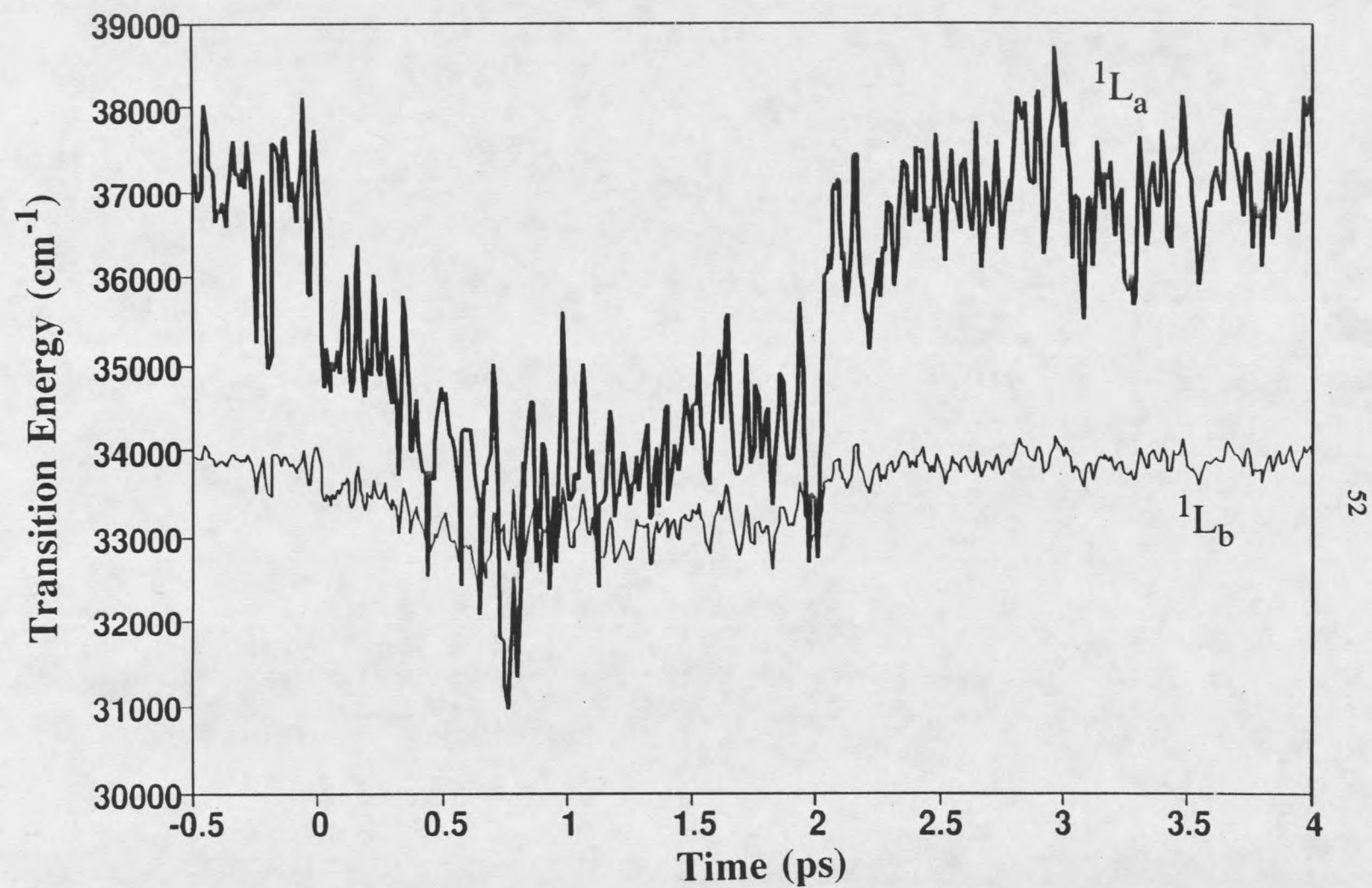


Figure 9. ¹L_a and ¹L_b transition energies for 3MI in water. The system absorbs a photon at t=0, and emits it at t=2 ps.

performed where the transition energies were obtained every femtosecond. It was discovered that all the structure could be observed if an INDO/S calculation was done every 10 fs, (5 fs for the first 200 fs after excitation), as reported in the METHODS section along with the time steps used with the other solvents. It was also clear that it was redundant to simulate excitation and emission, as this matched the former.

Another factor that was not taken into account was the difference in geometry between ground and excited states. This is not exceptionally important in order to calculate red shifts. However, in the case of 3MI, if the molecule remains in the ground state geometry, our calculations show that the 1L_a and 1L_b states are almost degenerate after excitation. In this situation, there is an avoided crossing between both states that complicates the interpretation of results. As a geometry for the 1L_a state has not been experimentally determined, we estimated it using an average of 4000 INDO/S calculations, each of them with a different solvent field. The procedure to obtain this geometry has been outlined in the METHODS section. This *ad hoc* geometry does not necessarily represent the 1L_a minimum, but it is displaced towards it, and this results in the 1L_a vertical transition being generally smaller than the 1L_b transition in polar solution. As an example of this decrease in transition energy, the values for the transition energies for 3MI in vacuum go from 33951 and 37185 cm^{-1} for 1L_b and 1L_a respectively, (S_0 geometry), to 33177 and 34071 cm^{-1} , (1L_a geometry). Figures 6 and 7 show the different geometries used.

Another refinement, dealing with the solute charges, was also introduced. In the calculation shown in Figure 9, the ground and excited state charges were calculated using

INDO/S for the isolated molecule, (vacuum). In principle, these values should not agree with values calculated for 3MI in solution. Thus, the 4000 INDO/S calculations used to calculate the 1L_a geometry were also used to obtain the charges for 3MI in solution.

3MI in Water

Fluorescence

In this section, (and similar ones for the other systems), we will show the results that yield information on the fluorescence red shift.

Figures 10 and 11 show results using the *semipolarizable* and *polarizable* procedure, respectively, (see METHODS section). Figure 10 shows the decay in the fluorescence, (as measured by the decay in the transition energies), for 3MI in water when the molecule is excited to the 1L_a state, averaged over 5 runs. In these simulations, the molecule is in the ground state from $t=-0.5$ to $t=0$ ps. At $t=0$, the solute is excited to the 1L_a state, in this case by changing its charges and its geometry from ground to 1L_a state values. The change in geometry can be seen in this figure in the vertical transition energy drop at $t=0$, (for both 1L_a and 1L_b states). It must be emphasized that this drop would also be present even in the absence of solvent because it is a purely geometric effect. Following the excitation, the solvent reorganizes as a consequence of the new dipole of the solute molecule, thus causing a red shift in the transition energies of both excited states. This similar behavior of both states' transition energies is expected because the red shift is related to the magnitude and orientation of permanent dipole of each state. Since the 1L_a dipole is larger than the 1L_b one and their orientation is roughly the same, it is

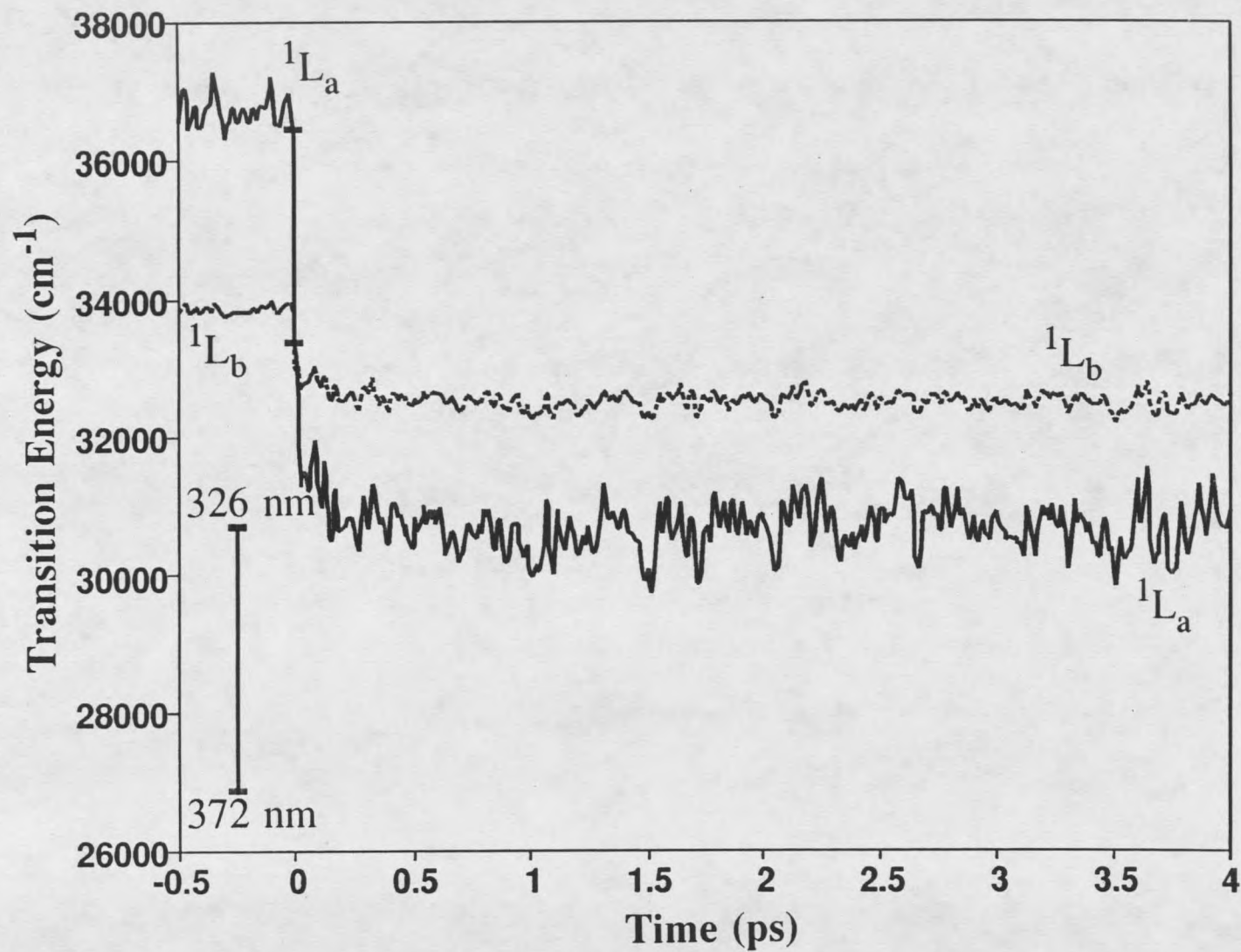


Figure 10. 1L_a and 1L_b fluorescence shifts for 3MI in water, (*semipolarizable*).

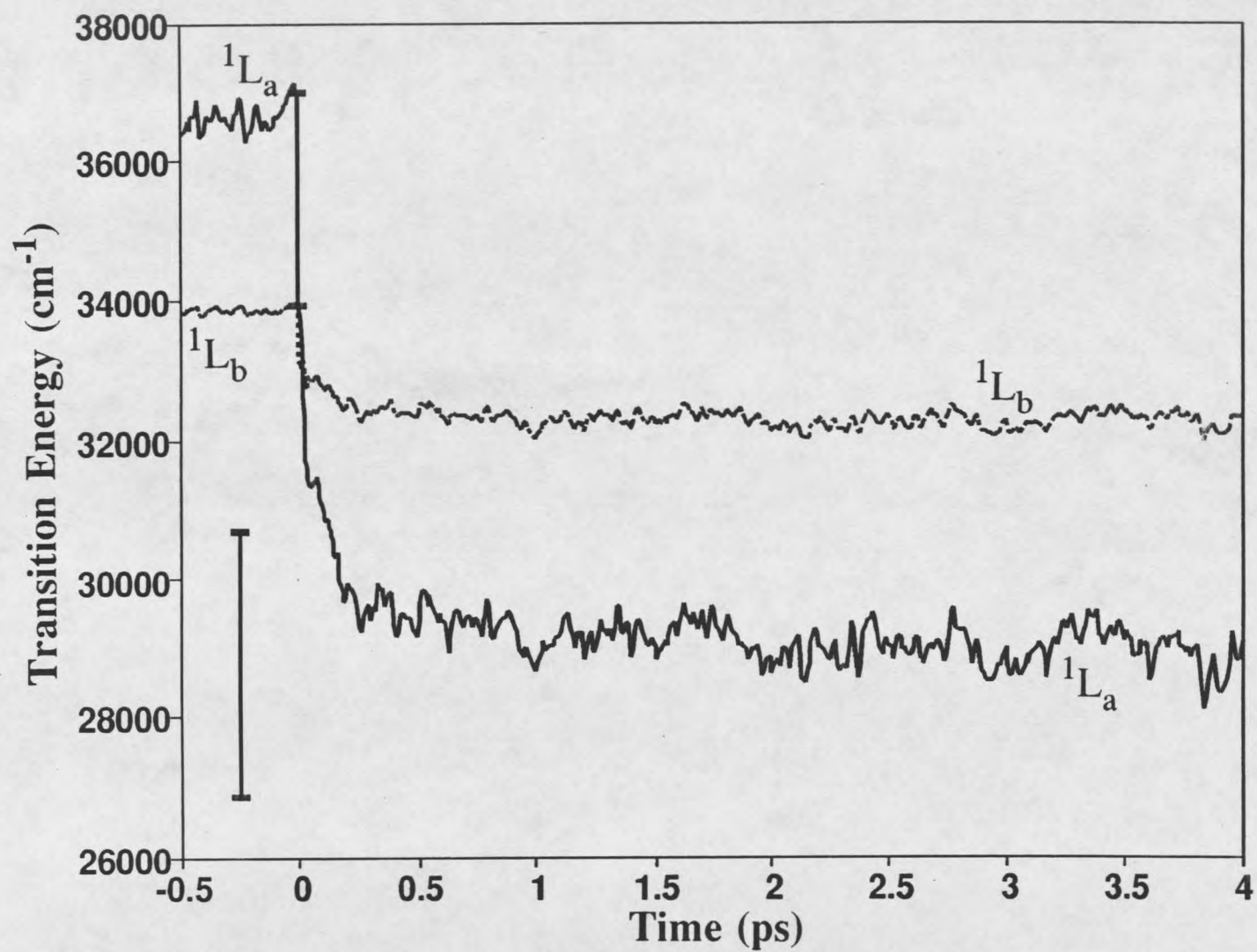


Figure 11. 1L_a and 1L_b fluorescence shifts for 3MI in water, (*polarizable*)

expected a very similar pattern for the transition energy decays with larger values for the 1L_a one.

The bracket at the bottom left of Figure 10 represents the experimental values^{131,132}. Unfortunately, no direct response has yet been measured directly for this system because the time scales involved here are too fast. Barbara *et al.*⁴³, and especially, Fleming *et al.*⁴⁴ have had success in measuring the subpicosecond response of coumarin dyes in water. Thus, this reported experimental shift, -3800 cm^{-1} , is compared to the difference between the fluorescence Stokes shift of a 3MI solution in water at 300 K ¹³¹, (where the solvent can reorient and stabilize the excited states after absorption), and that of a 3MI solution in an ethylene glycol-water glass at 140 K ¹³², (where, presumably, the solvent is frozen in its conformation and cannot stabilize the excited states after the excitation). However, this latter assumption does not seem to be completely true, (as will be shown later, for the simulations of 3MI in butanol at 0 K , relaxation by as much as 1000 cm^{-1} within a few picoseconds is possible in a glass). The experimental work of Lami *et al.*¹³³ on 1-methylindole and 2,3-dimethylindole in butanol at 118 K also shows that the solvent field around the emitting state is different from that previous to excitation. Therefore, this reported value of -3800 cm^{-1} can be considered a lower limit for the fluorescence shift. Our calculated value of $-2750\pm 750\text{ cm}^{-1}$ is significantly smaller. It is also noticeable that the absolute values for the initial and final transition energies are larger than the "experimental" values. However, this does not have any particular relevance because it is due to the fact that our calculated geometry for the excited state does not bring the solute to the excited state minimum, in which case the

absolute values for the transition energies would be smaller. At any rate, the important magnitude is the fluorescence shift, not the absolute values of the transitions.

In the calculation shown in Figure 10 the solvent is reacting to the new solute dipole after excitation. It is clear, then, that the environment seen by the 3MI molecule is changing. This should have the effect of inducing changes in the solute dipole, but, in Figure 10, we kept it fixed throughout the simulation.

The root of this behavior lies on the fact that the charges computed using INDO/S do not necessarily have to be the same ones used in MD. The latter ones are responsible for polarizing the solvent field, (the MD dipole of the solute is a solvent-field-*inducing* dipole). On the other hand, INDO/S calculates its own set of charges, which are going to be affected by the polarization of the solvent field, (thus, the dipole calculated by INDO/S is a solvent-field-*induced* dipole). In our calculations we can choose whether or not to make these dipoles equal. In the *semipolarizable* model just mentioned, the MD dipole has a fixed value, which happens to be ~35% smaller than the average of the INDO/S dipoles.

If the INDO/S calculated charges are input into MD, this would have the effect of polarizing the solvent field even more, which in turn would result in a larger calculated dipole in INDO/S, which would polarize the solvent field more, et cetera. This procedure, followed until self consistency, results in a slight increase, ~5%, in the calculated INDO/S dipoles, and a large increase, ~40%, in the dipoles used in MD. This is the basis of the *polarizable* model, which predicts larger red shifts as a result of the larger dipoles of the solute.

Figure 11 shows an average of 10 calculations using the so called *polarizable* model. In these calculations, every time INDO/S was used to obtain the excited state transition energies, the charges for the solute were also computed and used in the input for the next molecular dynamics steps, (see METHODS section). In this case, the average value for the solvent field inducing dipole, (1L_a state), went from 6.83 D in the "semipolarizable" model to 11.66 D, that is, an increase over 70%. The solvent induced dipole for the 1L_a state, (see above), went from 10.93 D to 11.66 D. Note that, as required by the self-consistency of this model, the solvent field induced and inducing dipoles are equal.

As a consequence of these larger dipole values, the calculated shift is also larger than in the previous simulation: $-4875 \pm 800 \text{ cm}^{-1}$ vs $-2750 \pm 750 \text{ cm}^{-1}$. In this case, the calculated value is larger than the "experimental" value of -3800 cm^{-1} , but it should be pointed out once again that this experimental value might be missing $\sim 1000 \text{ cm}^{-1}$ of subpicosecond fluorescence red shift.

Other calculations were done using the polarizable model and a different program, CHARMM, or a different geometry for the solvent cage, (a drop, as opposed to PBC). In both cases, the results were similar to those shown in Figure 11, although the calculated red shift was slightly larger, ($\sim -5500 \text{ cm}^{-1}$ for the CHARMM calculation and $\sim -5900 \text{ cm}^{-1}$ for the drop geometry). As the computational time required for these alternate calculations was larger, they were abandoned in favor of the previously described method, consisting in using Discover and PBC.

An interesting issue here is that of the time scale involved in the red shift. It can

be seen from Figure 11 that the relaxation is complete in less than 500 fs, but a good part of it takes place almost immediately after excitation. Figure 12 shows the first 1000 fs of the fluorescence decay shown in Figure 11. Almost half of the red shift takes place during the first 20 fs. In fact, the decay can be approximated by a Gaussian and an exponential decay.

$$v_f = 1680 \exp \left[- \left(\frac{t}{15.7 \text{ fs}} \right)^2 \right] + 3250 \exp \left(- \frac{t}{170 \text{ fs}} \right) + 29050 \text{ (cm}^{-1}) \quad (30)$$

In this case, 29050 cm^{-1} is the average value of the 1L_a transition energies after the solvent reorganization has taken place.

Using Equation 29, we can get an expression for $C_{eg}(t)$ for 3MI in H_2O .

$$\begin{aligned} C_{eg}(t) &= \frac{\left(1680 \exp \left[- \left(\frac{t}{15.7 \text{ fs}} \right)^2 \right] + 3250 \exp \left(- \frac{t}{170 \text{ fs}} \right) + 29050 \right) - 29050}{33980 - 29050} \quad (31) \\ &= 0.341 \exp \left[- \left(\frac{t}{15.7 \text{ fs}} \right)^2 \right] + 0.659 \exp \left(- \frac{t}{170 \text{ fs}} \right) \end{aligned}$$

Such fast response is a little puzzling. What can be happening so fast?. The fact that the decay can be approximated by a Gaussian for the fast time scale suggests that the response is inertial in character^{46,48,49,134}. To verify this hypothesis, we have analyzed the first 20 fs of each of the 10 calculations averaged in Figures 11 and 12. Each of those 10 cases was compared to an equivalent calculation, (with the same starting point),

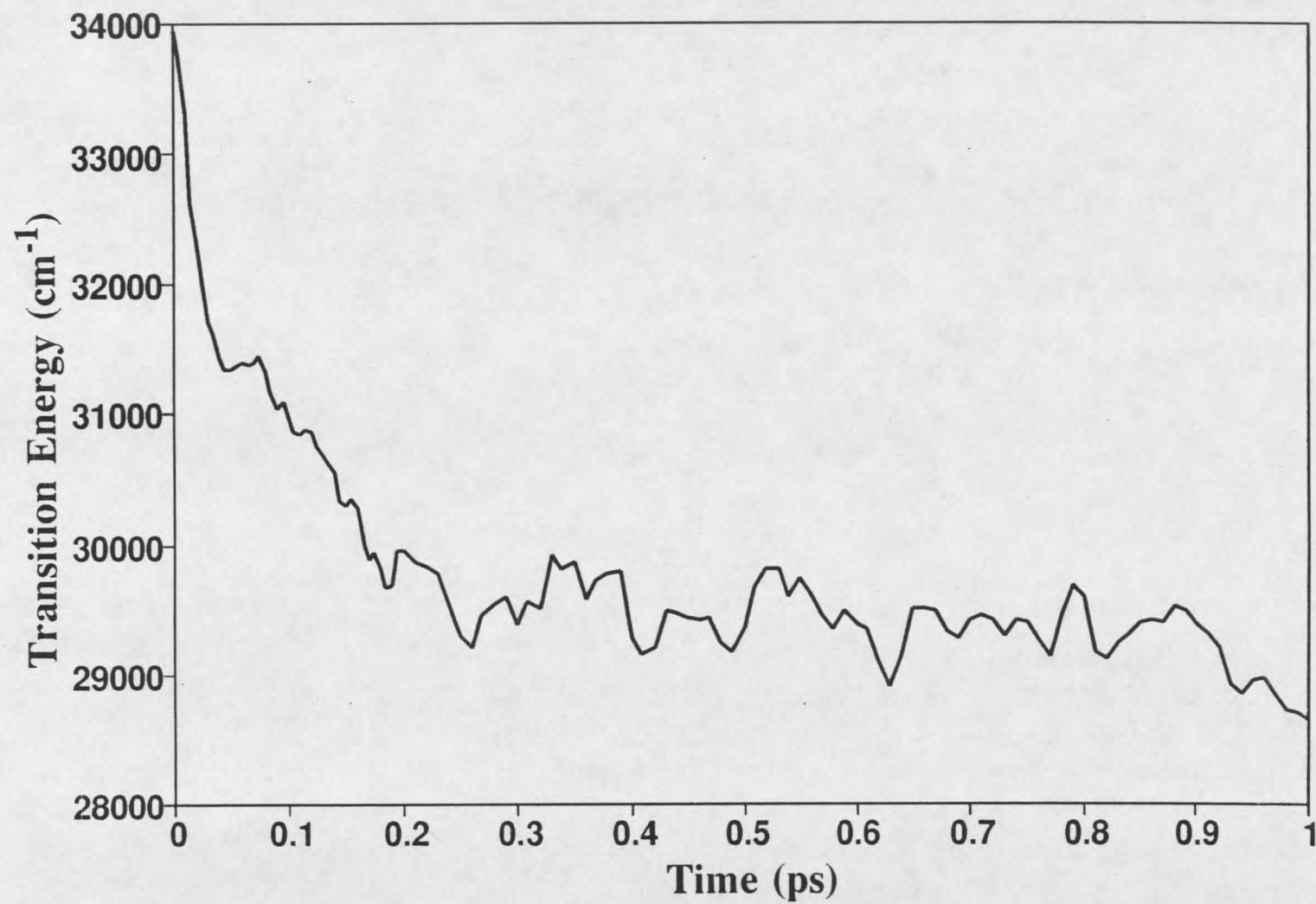


Figure 12. Zoom of Figure 11 for the first 1000 fs after excitation.

but where the solute was never excited to the 1L_a state. Thus, the solvent motion in this second set of calculations was purely thermal, as opposed to the simulations where the molecule was excited, in which the solvent motion had not only a thermal component, but also was partly due to a new solute electrostatic field. If the thermal motion were subtracted, the residual displacements could be assigned to the change in the solute dipole. Figure 13 shows a snapshot of two equivalent trajectories taken at $t=20$ fs. For most of the solvent molecules, all the observable motion is purely thermal in nature, as evidenced by the fact that each two equivalent molecules superimpose so well that we can see only one. But there are a few cases where there is a significant, albeit small, displacement.

This motion is purely rotational in the sense that the center of masses always moves less than $0.005 \text{ \AA}$. Even the rotational motion is small, $<20^\circ$ for these few molecules, $\sim 1^\circ$ in average, (as opposed to $\sim 35^\circ$ average for the thermal motion between $t=0$ and $t=20$ fs), but it accounts for a significant fraction of the red shift. Maroncelli⁴⁶ has shown, for monoatomic ions in acetonitrile, that the fast response is not only inertial in character, but also that each molecular response is independent of the others: the total response is equivalent to the sum of the responses when only one molecule is allowed to move and the others are fixed in place.

Figure 14 shows a plot of the rotational contribution to the red shift as a function of the distance to the solute, (at $t=20$ fs). It can be seen that only molecules closer than $\sim 6 \text{ \AA}$ have an effect on the red shift.

At $t=150$ fs, most of the relaxation has taken place. When a procedure similar to the one explained for $t=20$ fs is applied, the results are not so simple. The rotational

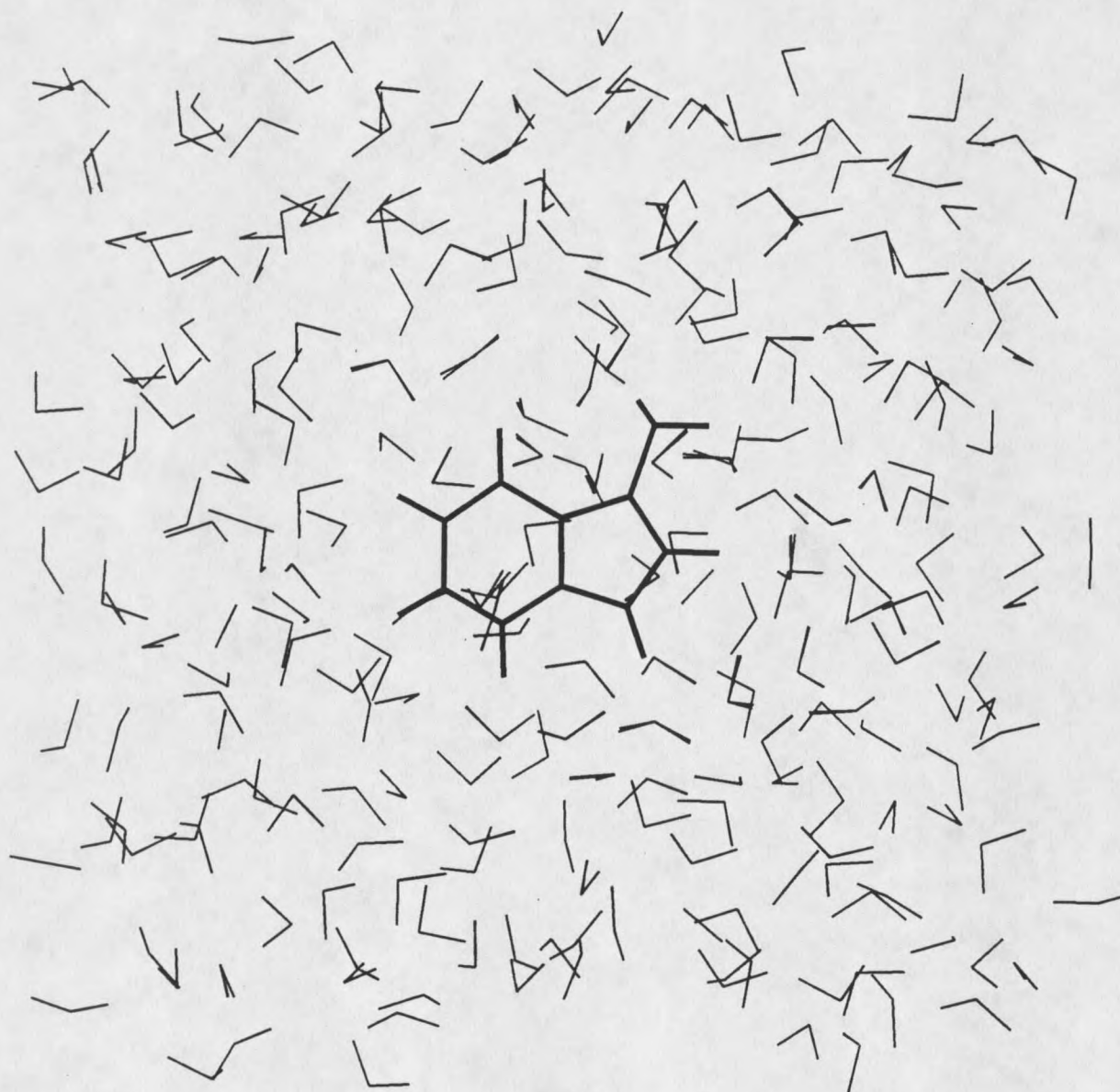


Figure 13. Superimposed snapshots of two trajectories 20 fs after excitation.

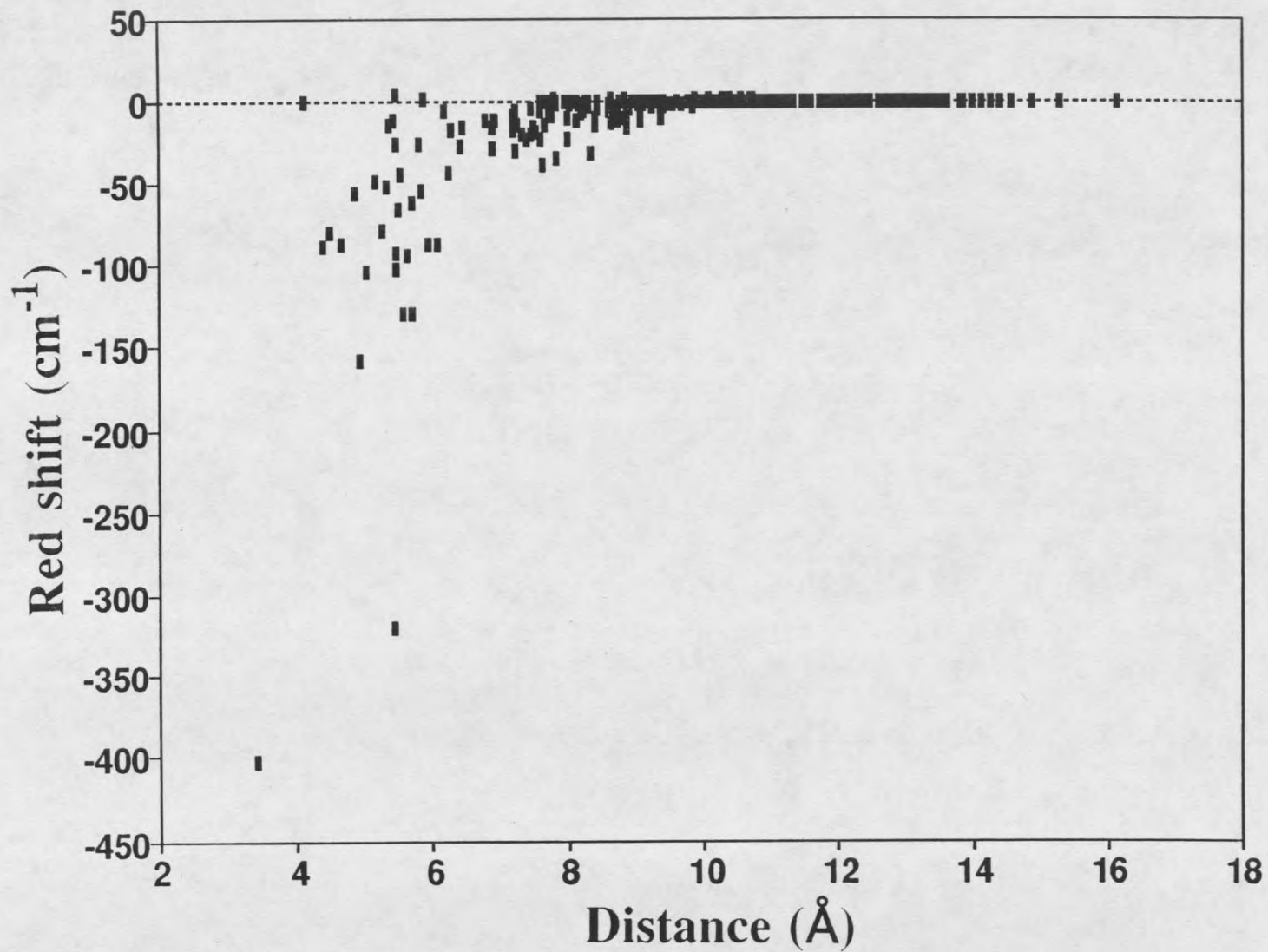


Figure 14. Rotational contribution to the red shift vs distance, for the same trajectories shown in Figure 13.

contribution is still dominating, but most of the molecules show a displacement of the center of mass that accounts for ~20% of the red shift. It is important to note that those molecules responsible for the largest amount of red shift at $t=20$ fs are not the same ones responsible for the main contribution to the red shift at $t=150$ fs. This suggests that at any given time the main contribution to the red shift comes from those molecules that, because of their position, can align their dipole with that of the solute by a fast rotational motion of their hydrogens. This also seems to dispel the necessity of formation of excited state complexes, (exciplexes), to explain the red shift^{2,5}. Figure 15 shows a picture of 3MI with 13 solvent molecules, (from 10 different simulations), each of which, at $t=150$ fs, contributes between 1500 and 4000 cm^{-1} to the fluorescence red shift. It can be seen that they are located in completely different places over the molecule, providing evidence against a specific site for exciplex formation.

There is some organization in Figure 15. Those solvent molecules over the five-membered ring point their oxygen towards the ring, while those over the six-membered ring point their oxygen away from the ring, thus favoring the electron transfer towards the six-membered ring which is the signature of the transition to the 1L_a state.

Although the notion of exciplex formation does not appear to be needed to explain the red shift, it is not surprising that the solvent cloud resulting from the solvent being in equilibrium with the solute ground state is different from the cloud formed when the solvent is in equilibrium with the solute 1L_a state. Figure 16 shows the radial distribution, (RDF), (normalized to 252 water molecules), of solvent hydrogen atoms around the C4 atom of 3MI for ground and excited states. The hydrogen cloud gets much closer to the

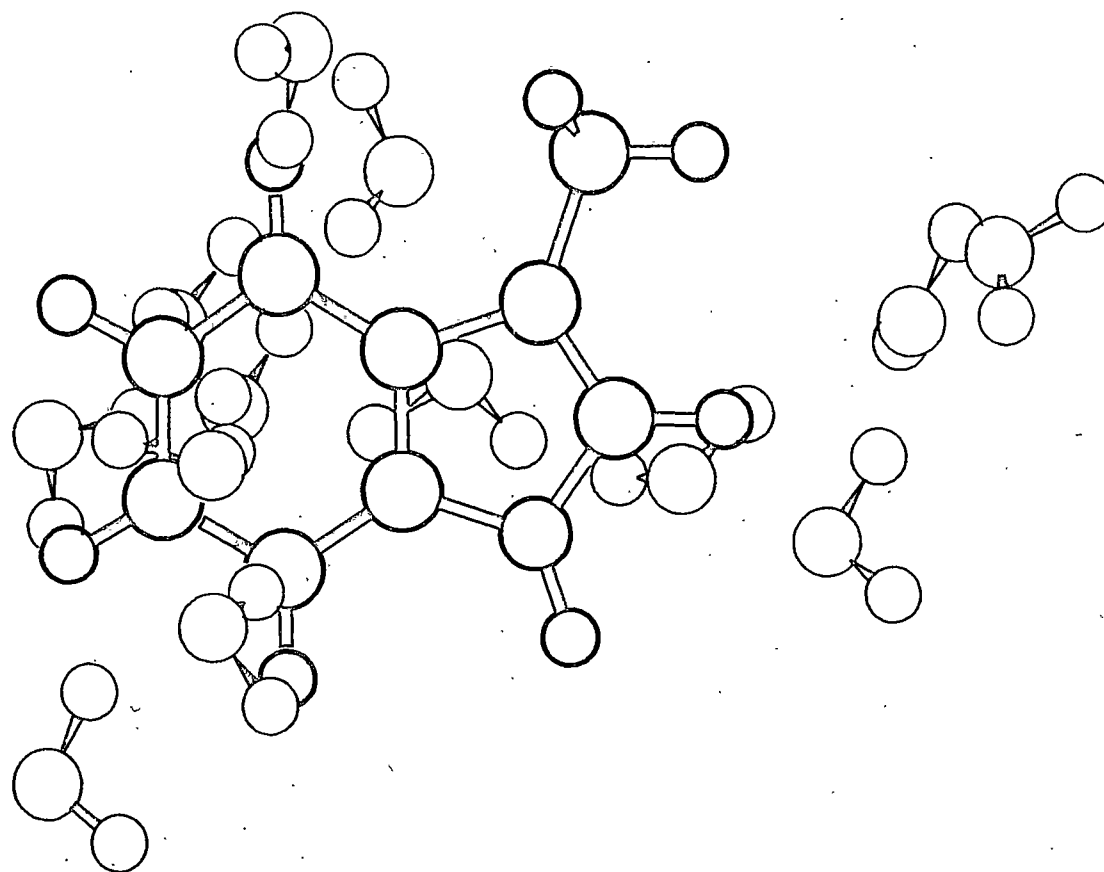


Figure 15. 3MI with 13 water molecules which make exceptional contributions, ($>1500\text{cm}^{-1}$), to the fluorescence red shift at $t=150$ fs after excitation, (from 10 different trajectory pairs of the type displayed in Figure 13).

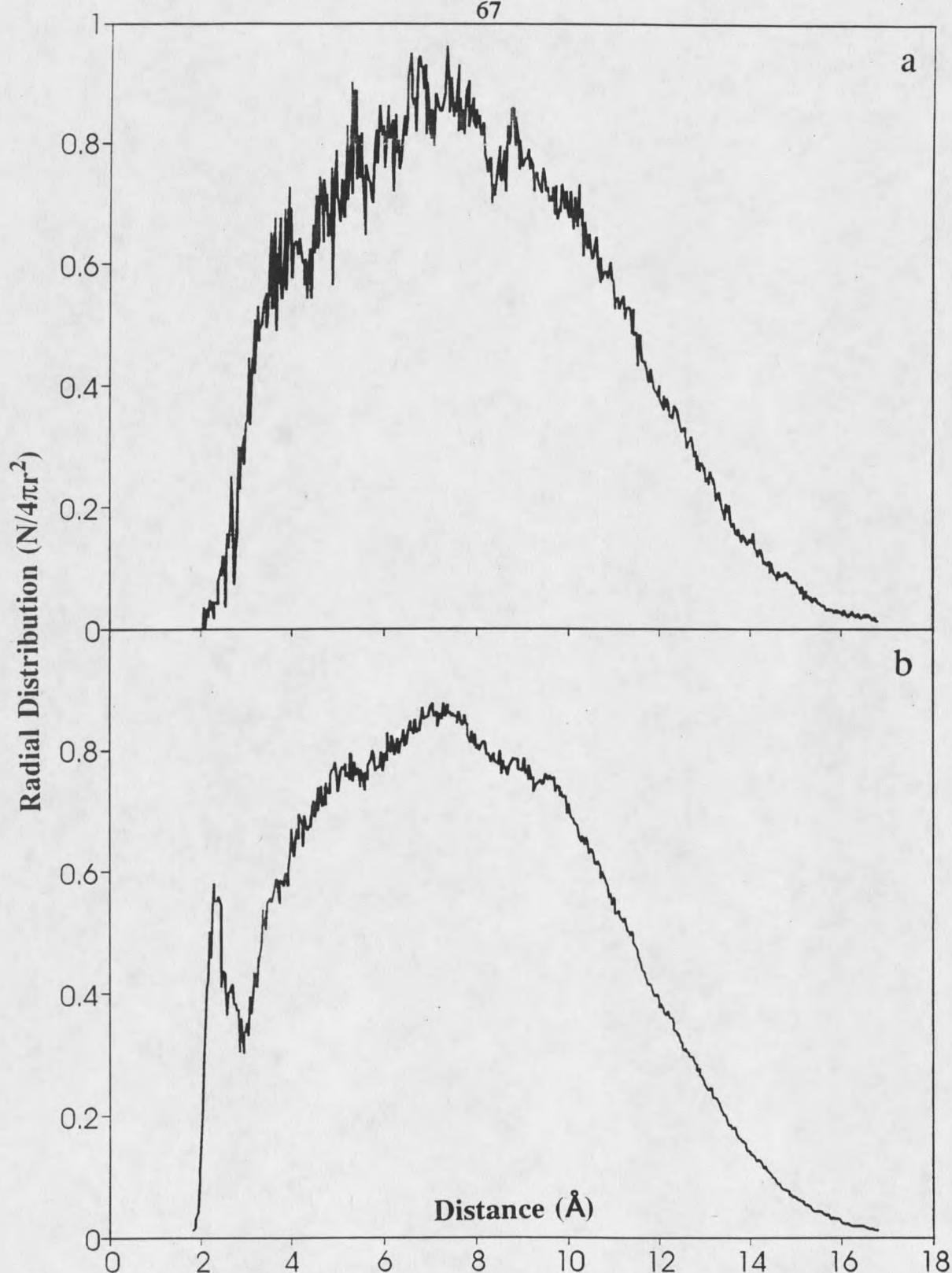


Figure 16. Radial Distribution Functions for the distance from C4 to the hydrogen atoms in the water molecules, for the ground, (a), and 1L_a , (b), states of 3MI.

carbon atom when the solute is in the 1L_a state, which is not surprising, considering that C4 receives a large part of the electron transfer. Integration of the region between 1.8 and 2.4 Å, (taking into account that the radial distributions in Figure 16 have been divided by $4\pi r^2$), indicates that the number of water molecules increases from 0.06 to 1.0 following excitation and equilibration, thus supporting the idea of hydrogen-bond formation, after the solvent has relaxed. The hydrogen bond length of 2.4 Å, (the distance corresponding to the maximum of the radial distribution function), however, will not be particularly strong, because it is much longer than that found in water^{135,136}, 1.76 ± 0.04 Å, and about the same distance as the weak π H-bond found between water and benzene¹³⁷.

Figure 17 shows the RDF for the distance to the oxygen atom in the solvent molecules for three different atoms in 3MI: N1, (a), C2, (b), and H1, (c). The first two suffer a loss of 0.15-0.20 e^- upon excitation and are expected to attract the oxygen atoms closer. Indeed, it can be seen in the figure, that the RDF is slightly closer for the excited state, (in thick line). Integration of the functions also reveal this phenomenon. If we define a distance, r_1 , corresponding to the radius of a sphere containing an average of 1 atom of oxygen, (or hydrogen), from the solvent molecules, r_1 is 3.27 Å for N1 when 3MI is in the 1L_a state, and 3.36 Å in the ground state. These numbers are 3.39 Å and 3.51 Å, respectively, for C2. There is another atom in 3MI, (C3), which also loses electron density upon excitation, but no change in the RDF is observed, probably because short range interaction with the solvent molecules is hindered by the methyl group.

H1 does not lose or gain any charge upon excitation in our calculations. However,

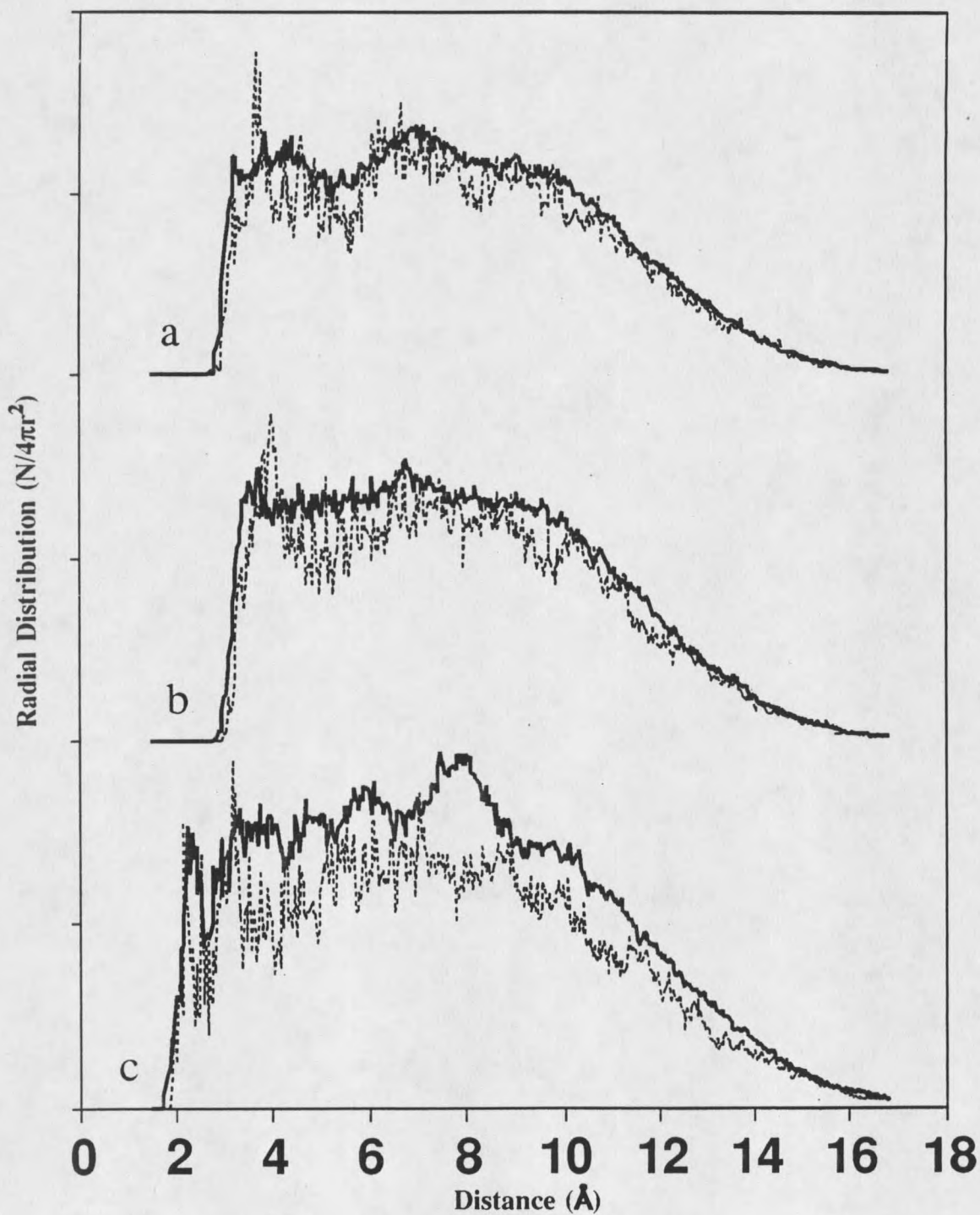


Figure 17. Radial Distribution Functions for the distance from N1, (a), C2, (b), and H1, (c), to the oxygen atoms in the water molecules for the ground, (thin dotted line), and ¹L_a states, (thick solid line), of 3MI.

it is interesting to examine the behavior of the RDF since a hydrogen bond can be expected at this position because of the higher positive charge of H1 as compared to the other hydrogens in 3MI. Furthermore, the charge loss at N1 can be interpreted as corresponding to the N-H unit, and can be felt by oxygens beyond H1. On an average, the oxygen atoms do get a little closer: r_I is 2.67 Å for the 3MI in the excited state vs 2.76 Å for 3MI in the ground state.

Figure 18 shows the RDF for the hydrogen atoms around C5, (a), and C7, (b), which received a large portion of the electron transfer. It can be easily seen that the hydrogen atoms get much closer to C5 or C7 after excitation. In C5, r_I is 2.49 Å, (almost the same value as for C4), for the 1L_a state, and 2.94 Å for the ground state. For C7, these values are 2.73 and 3.06 Å.

From these numbers shown, it seems clear that the different charge patterns between ground and excited states causes a reorganization of the solvent around the solute. It must be pointed out, however, that all these phenomena are observed after the fluorescence shift has taken place. It is also observed that, when an atom receives electron transfer, the displacement of the hydrogen cloud towards the atom is larger than the displacement of the oxygen cloud towards an atom which lost electronic character.

Similar calculations to those shown in Figures 11 and 12 were performed for 3MI in D₂O. A similar behavior as that calculated for 3MI in H₂O was expected, except for the relaxation time of the Gaussian component. The results agree with these expectations, although the calculated red shift is some 15% larger than the 3MI-H₂O red shift, (-5700±800 vs -4875±800 cm⁻¹). The difference in these two estimates indicates that the

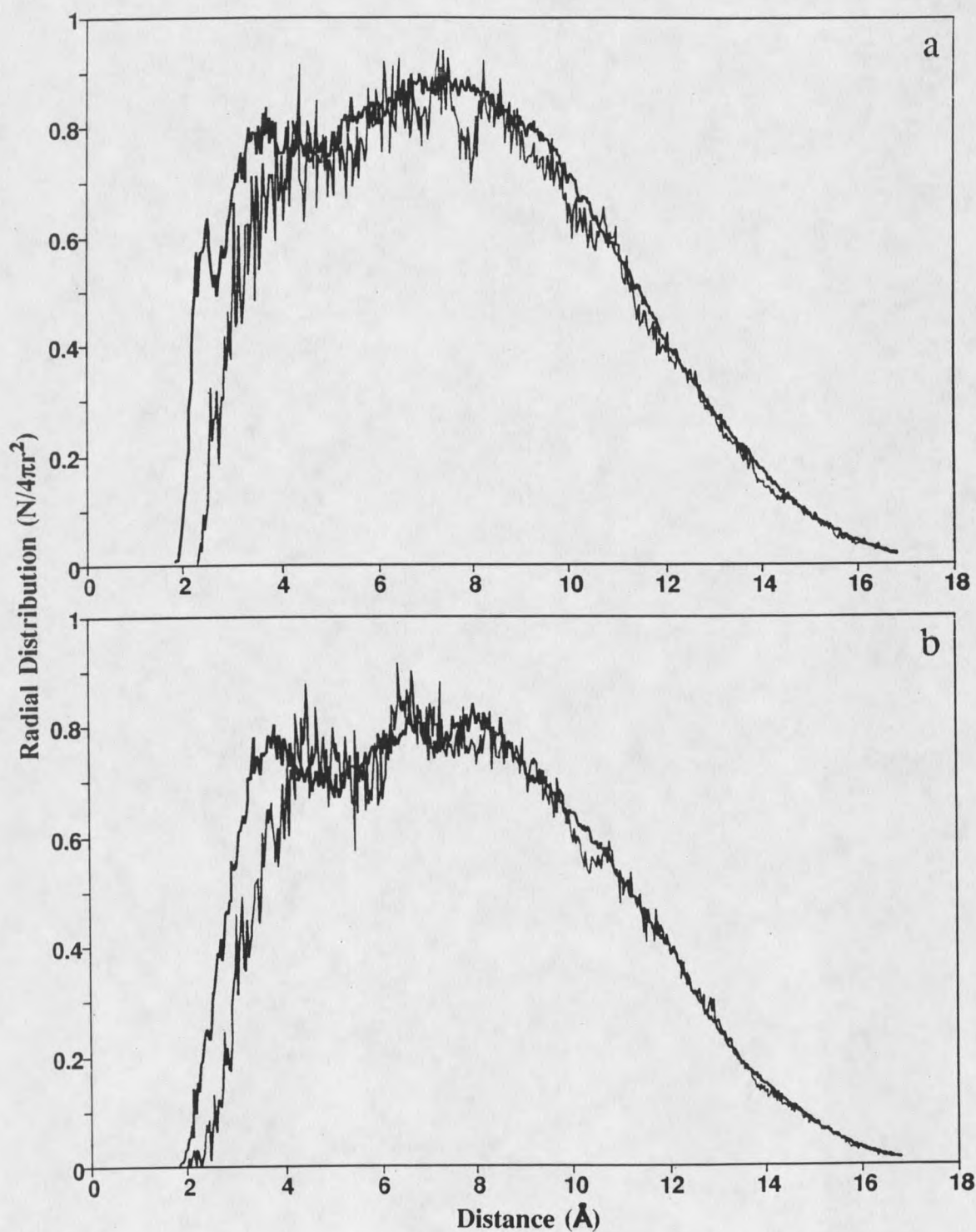


Figure 18. Radial Distribution Functions for the distance from C5, (a), and C7, (b), to the hydrogen atoms in the water molecules for the ground, (thin line), and 1L_a states, (thick line), of 3MI.

error in our calculations is large, since a differential shift between H_2O and D_2O is not expected. The overall decay can be approximated by the following function:

$$v_f = 2115 \exp \left[- \left(\frac{t}{25.1 \text{ fs}} \right)^2 \right] + 3600 \exp \left(- \frac{t}{225 \text{ fs}} \right) + 26550 \text{ (cm}^{-1}) \quad (32)$$

Thus, $C_{eg}(t)$ for 3MI in D_2O is:

$$\begin{aligned} C_{eg}(t) &= \frac{\left(2115 \exp \left[- \left(\frac{t}{25.1 \text{ fs}} \right)^2 \right] + 3600 \exp \left(- \frac{t}{225 \text{ fs}} \right) + 26550 \right) - 26550}{33265 - 26550} \quad (33) \\ &= 0.370 \exp \left[- \left(\frac{t}{25.1 \text{ fs}} \right)^2 \right] + 0.630 \exp \left(- \frac{t}{225 \text{ fs}} \right) \end{aligned}$$

The relative amplitudes of the Gaussian and exponential decays are very similar to that for H_2O . However, the important issue here is that of the relative magnitudes of the Gaussian relaxation time. τ_G is 15.7 fs for H_2O and 25.1 fs for D_2O . Rothschild¹³⁸ has suggested that the ratio of τ_G 's should be proportional to the square root of the ratio of the moments of inertia of the two solvent species when the behavior is inertial. In this case, this ratio is 1.39. Our calculated ratio, 1.6 ± 0.1 , disagrees with this previous statement, but agrees with previous calculations, reported by Perera and Berkowitz⁴⁸, and Neria and Nitzan⁴⁹, as pointed out by the analysis by Bagchi and Chandra¹³⁴, where the ratio of τ_G 's is intermediate between that predicted by Rothschild and the ratio of moments of inertia, 1.93.

Absorption

Information about the absorption red shift induced when going from vacuum to solution and about the associated inhomogeneous broadening can be easily calculated using this method. However, unlike in the simulations reported in the previous section, the solute molecule does not need to be excited to the 1L_a state. The absorption maximum in solution can be obtained by leaving the system in the ground state and calculating the average of the transition energies for a large number of solvent conformations. The red shift is then obtained by subtracting the transition energy in vacuum. A frequency distribution of the transition energies is Gaussian in shape, (if enough conformations are used), and its width gives information about the broadening.

Figure 19 shows the transition energy fluctuations for both 1L_a and 1L_b states over a period of 30 ps. Energies and dipoles are calculated every 5 fs. The new charges are used as input for the next molecular dynamics steps. Therefore, the solute is *polarizable*. The horizontal lines in Figure 19 represent the calculated transition energies for the isolated 3MI molecule (vacuum). It can be seen that during most of the simulation the solvent field red shifts the transition energies with respect to vacuum, but there are some conformations that cause a blue shift. All these different microenvironments are responsible for the broadening. It is also observed that a few solvent environments bring the 1L_a state below 1L_b , even at the ground state geometry, (the transition energies shown here correspond to the vertical transitions). This implies that the 1L_b equilibrium geometry, which is very similar to that of the ground state, (see Figures 6 and 7), is above the 1L_a origin for 3MI in water, because the 1L_a origin is, at least, 3000 cm^{-1} below the

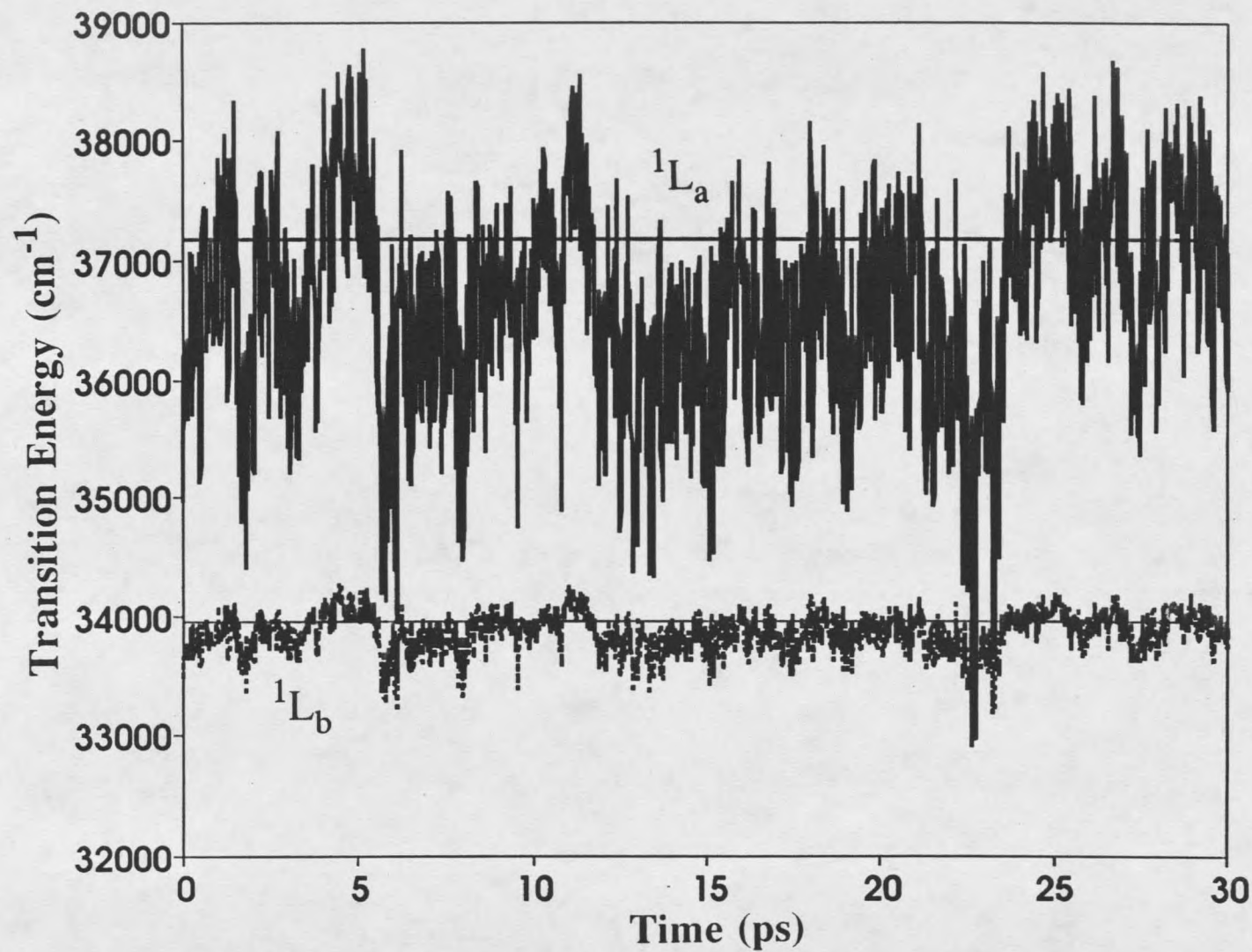


Figure 19. 1L_a and 1L_b transition energy fluctuations for 3MI in water.

vertical energy calculated at the S_0 geometry, (see Figure 11).

These large fluctuations in the transition energies seem to indicate that the oscillations in the permanent dipole moments are also large. Figure 20 shows a plot of the permanent dipole of the 1L_a state, (at the ground state geometry), as a function of the 1L_a transition energy. We can see that the permanent dipole changes along a large range: from ~ 3 D at 39000 cm^{-1} to ~ 12 D at 33000 cm^{-1} . This large polarizability of the 3MI molecule with respect to the solvent explains the difference in calculated fluorescence red shift for the two models shown in the previous section, (see Figures 10 and 11).

The average transition energies for the 1L_a and 1L_b states are 36635 and 33870 cm^{-1} respectively, which represent an absorption red shift of $-550 \pm 300\text{ cm}^{-1}$ for 1L_a and $-80 \pm 40\text{ cm}^{-1}$ for 1L_b . This latter value compares rather well with the experimental value for the 1L_b absorption shift of -95 cm^{-1} ¹³. However, the experimental value for the 1L_a state is much larger, -1450 cm^{-1} ¹³, than the calculated value. The reason for this poor agreement will be discussed later, when these results are compared to the calculated absorption shifts for indole in water.

Figure 21 shows a frequency distribution of the 6000 calculated transition energies for both states, (not normalized for different oscillator strengths). The size of the intervals used to calculate the frequencies were 50 and 200 cm^{-1} for 1L_b and 1L_a respectively. The shape of the distribution is Gaussian, as expected for the inhomogeneous limit of the broadening, as it is in this case, where the different conformations generated along the time coordinate can be considered equivalent to different spatial configurations, (microstates). The full width at half maximum, (FWHM), is $400 \pm 50\text{ cm}^{-1}$ for 1L_b and

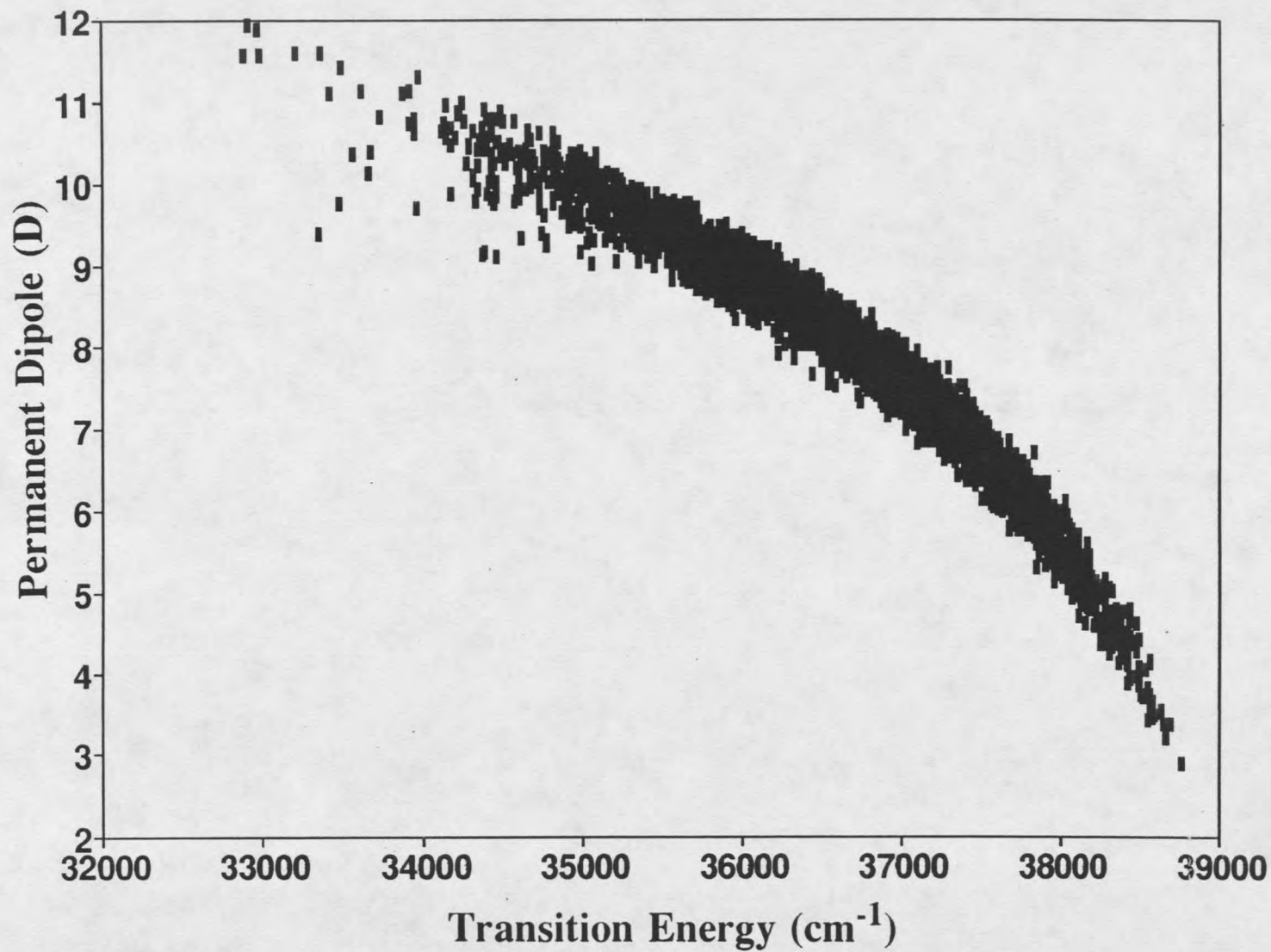


Figure 20. 1L_a Permanent Dipole vs Transition Energy, (for the 6000 configurations in Figure 19).

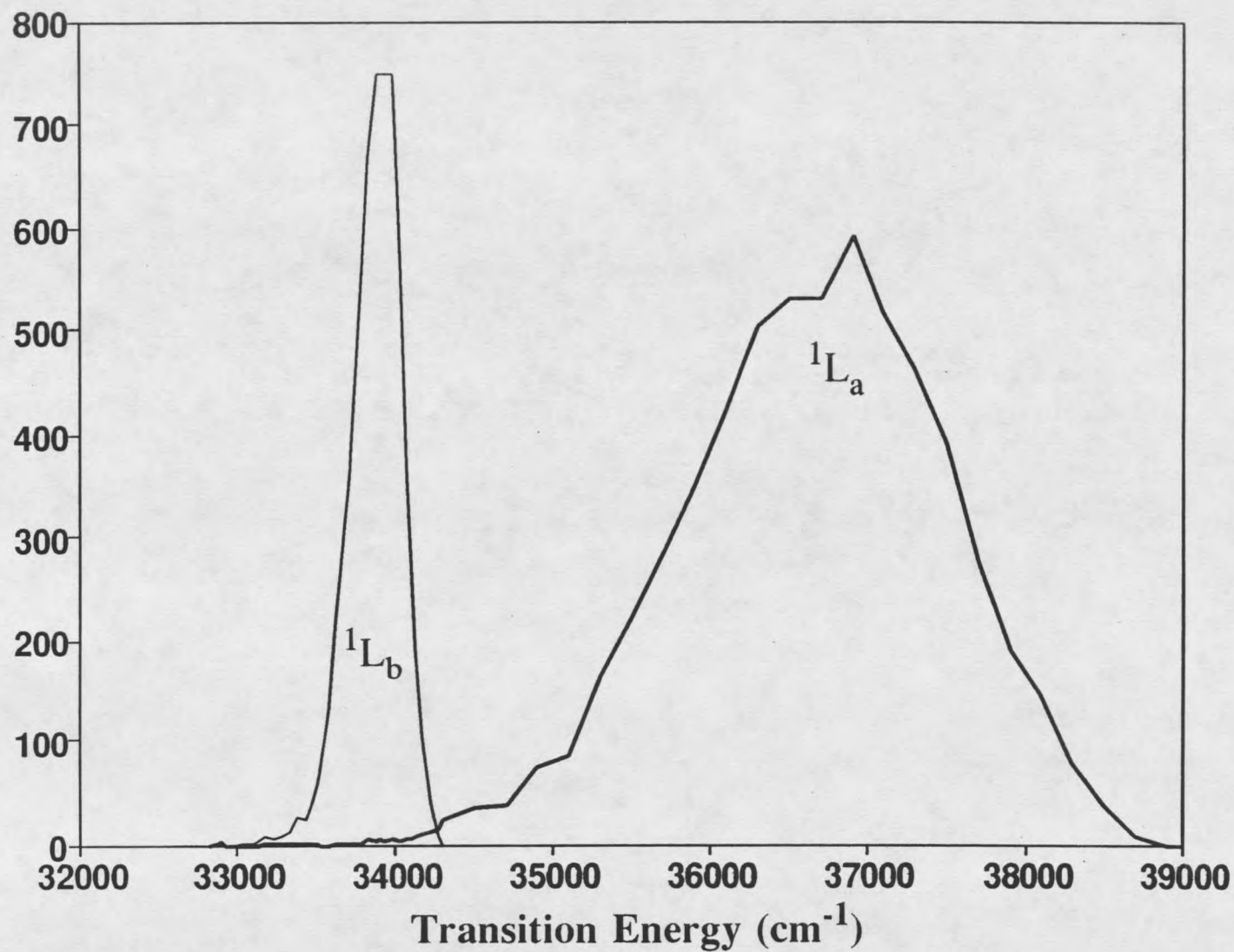


Figure 21. Distribution of the transitions energies shown in Figure 19.

$2000 \pm 100 \text{ cm}^{-1}$ for 1L_a . Demchenko *et al.*¹³⁹ have deduced an experimental width of 1040 cm^{-1} for indole in glycerol, which is in qualitative agreement with our result, especially if we consider the smaller dipole of indole, as compared to 3MI, and the smaller dielectric constant of glycerol¹³, as compared to water. It can be understood from Figure 20 why smaller fluctuations would result in smaller widths.

Similarly, for 3MI in D_2O , the calculated absorption shifts are $-750 \pm 450 \text{ cm}^{-1}$ for 1L_a and $-150 \pm 40 \text{ cm}^{-1}$ for 1L_b , with the linewidths being $2200 \pm 200 \text{ cm}^{-1}$ and $400 \pm 50 \text{ cm}^{-1}$, respectively. The results are similar to those reported previously for 3MI in H_2O , (if we consider the error bars), as it was expected.

From Figure 19, we can also extract information about the fluorescence red shift. As pointed out by Bagchi and Chandra¹²⁵, the autocorrelation function of the transition energy fluctuations, (see Equation 29), should be equivalent to the calculated direct response, (as shown in Figure 11). In practice, they are not quite the same, because of numerical error, (since we are using a finite number of configurations), and because the solute geometries are different, (the direct response was calculated for the 1L_a geometry while the autocorrelation function of the transition energy fluctuations was calculated with the molecule in the ground state). Figure 22 compares the calculated autocorrelation function with the direct response for the first 1000 fs. Both trajectories compare fairly well. The autocorrelation function can be also approximated as a linear combination of a Gaussian and an exponential decay:

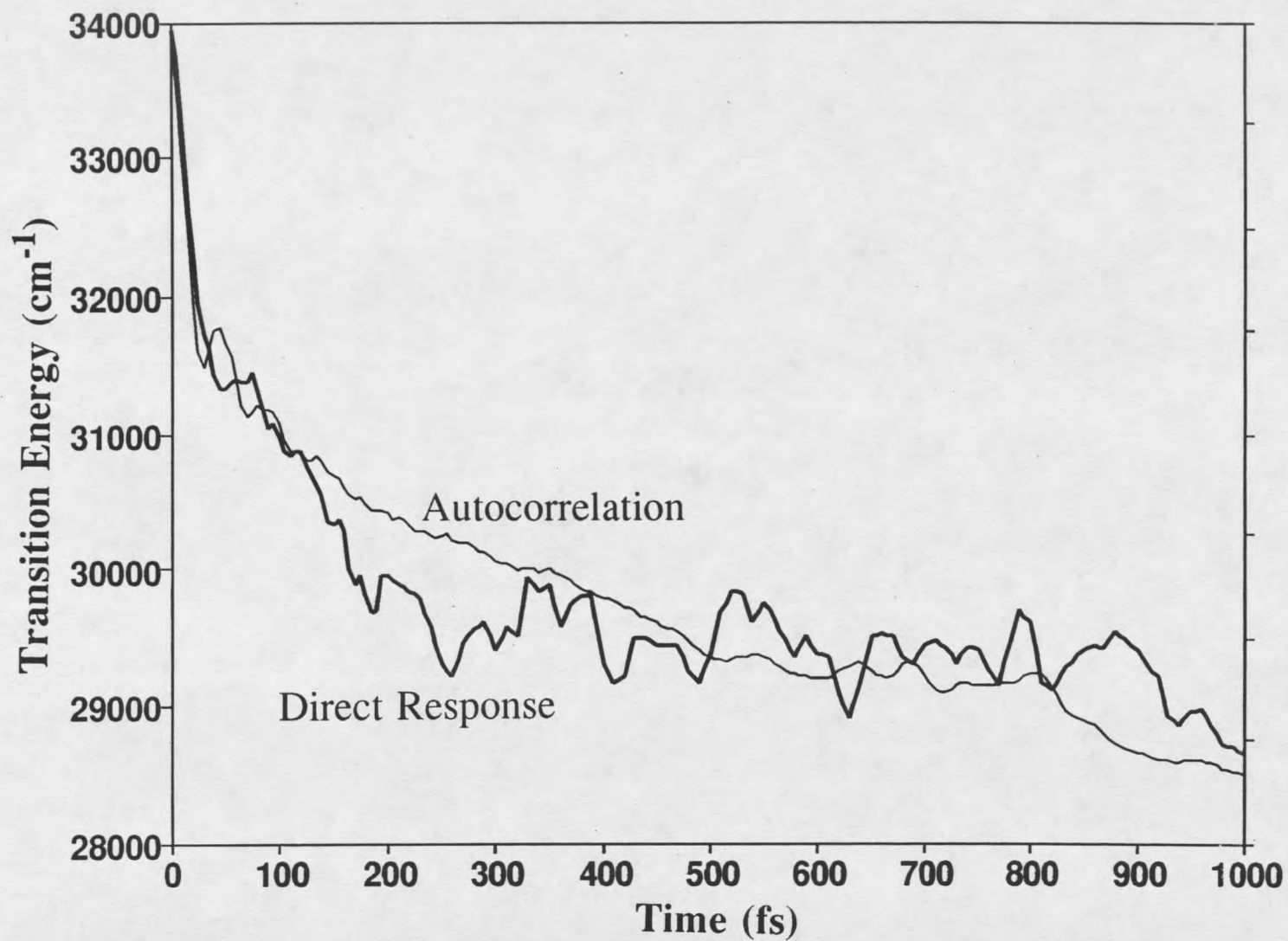


Figure 22. Autocorrelation of the ${}^1\text{L}_a$ transition energy fluctuations, (from Figure 19), and direct fluorescence shift, (from Figure 11), for 3MI in water.

$$C_{eg}(t) = 0.389 \exp \left[- \left(\frac{t}{15.2 \text{ fs}} \right)^2 \right] + 0.610 \exp \left(- \frac{t}{410 \text{ fs}} \right) \quad (34)$$

In comparing Equations 31 and 34 we find some interesting similarities: the fast response, (Gaussian), is almost identical in both cases, ~15 fs, while the exponential decay is rather different, 170 fs vs 410 fs. The magnitude of the Gaussian decay is significantly smaller in both cases than the magnitude of the exponential decay.

For D₂O, the calculated autocorrelation function can be approximated by

$$C_{eg}(t) = 0.297 \exp \left[- \left(\frac{t}{20.2 \text{ fs}} \right)^2 \right] + 0.703 \exp \left(- \frac{t}{420 \text{ fs}} \right) \quad (35)$$

which, once again looks very similar to its H₂O counterpart, (Equation 34), except for the calculated Gaussian correlation time. The ratio of these τ_G 's in this case matches that predicted by Rothschild¹³⁸, that is the square root of the ratio of the respective moments of inertia.

Indole in Water

Fluorescence

Figure 23 shows the time resolved fluorescence decay for indole in water. As a result of the larger INDO/S calculated dipoles as compared to 3MI, (13.0 vs 11.7 in average), the calculated red shift is also larger, -6000 cm⁻¹, which is ~1100 cm⁻¹ more

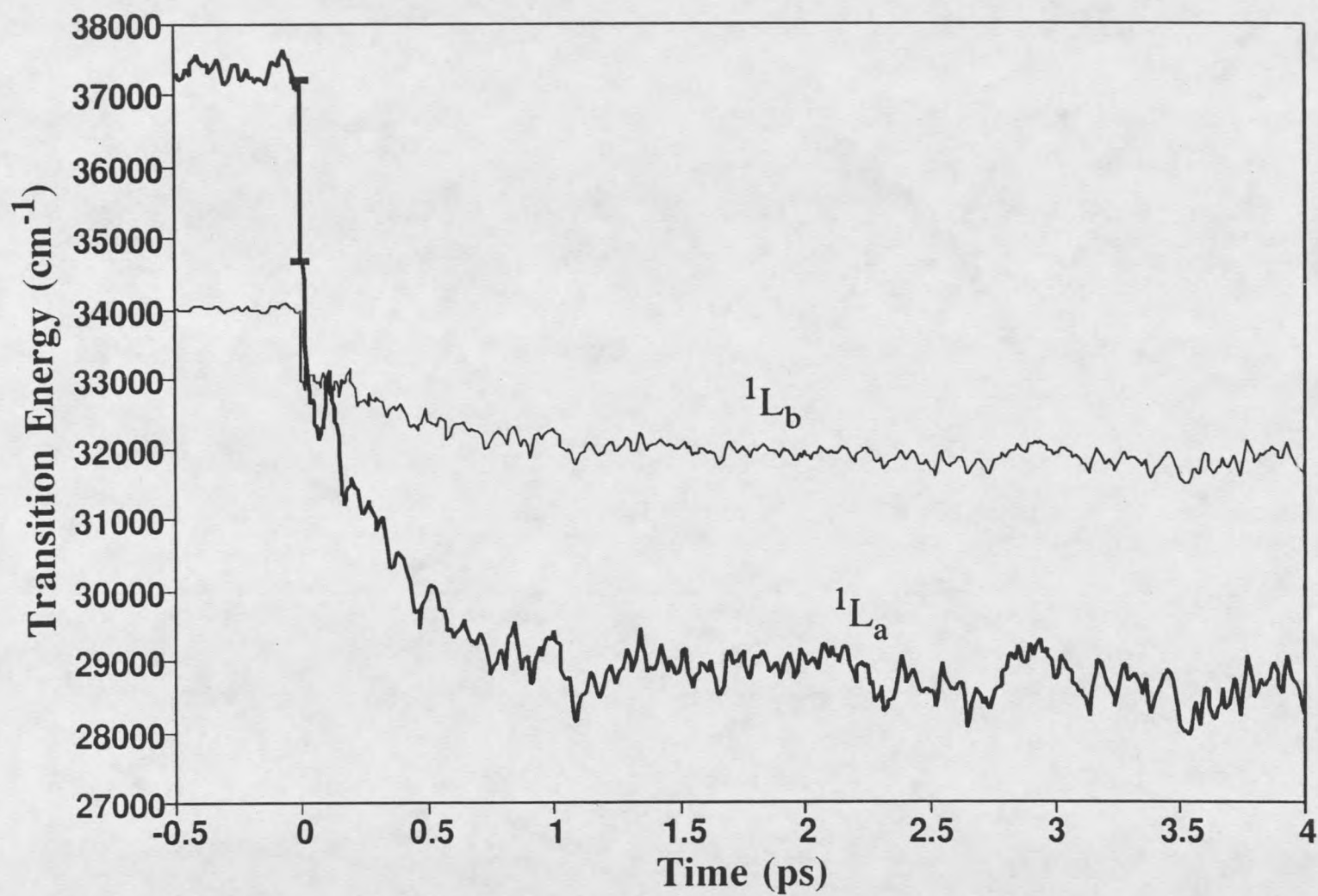


Figure 23. 1L_a and 1L_b fluorescence shifts for indole in water.

than calculated for 3MI in water.

This value is much larger than the experimental value, deduced from the work of Eisinger and Navon³, of -3500 cm^{-1} . It seems that the dipoles calculated by INDO/S are anomalously large. Similarly, the work of Callis¹²⁰ suggests that INDO/S predicts very well the two-photon properties of 3MI, but is not so good for indole.

The fast response Gaussian decay has a correlation time of 15.3 fs, which is comparable, (as expected), to that calculated for 3MI in water. The slow response has a rather large correlation time, (390 fs), and its amplitude is 3 times that of the fast response.

As it was done for 3MI in water, the fluorescence decay was also simulated using the *semipolarizable* model, which predicted a red shift of -2750 cm^{-1} . Once again, this model predicts smaller than experimental values.

Testing the Onsager-Mataga-Lippert Formulation

At this point, it was obvious that the calculated dipoles had a decisive influence in the calculated red shift. The Onsager-Mataga-Lippert model^{84,87-93} predicts that the relation between the fluorescence and absorption shifts for a molecule in a spherical cavity of radius a , inside a solvent of dielectric constant ϵ and refractive index n is given by Equation 19.

To test the validity of this formulation, we performed several calculations on the indole-water system using the *semipolarizable* model. In these calculations, the solvent reaction field-inducing permanent dipoles for both ground and excited states of the solute were scaled by different factors ranging from 62.5% to 150% of the calculated values, in

steps of 12.5%, for a total of 8 sets of simulations. Each of these sets consisted of 5 runs, which were subsequently averaged together. $\Delta\bar{v}_a$ was calculated, in each case, as the difference between the average value of the 1L_a transition energies, (with the solute in the ground state), and the 1L_a transition energy for the solute in absence of solvent. Similarly, $\Delta\bar{v}_f$ was calculated as the difference between the average value of the 1L_a transition energies measured once the relaxation was completed, and the transition energy for the solute, (in the 1L_a geometry), in the absence of solvent.

Figure 24 shows a plot of the calculated $\Delta\bar{v}_f - \Delta\bar{v}_a$ values, (note the change in order with respect to Equation 18, which results in a positive slope), vs $\Delta\mu'_{eg}\mu'_e - \Delta\mu_{eg}\mu_g$, and an adjusted linear regression fit for the first six points. The fit was obtained setting the y-intercept equal to 0. The regression shows a reasonable fit, with $r^2=0.976$. The point farthest to the right corresponds to the *polarizable* model.

By assuming $\epsilon=80$, the experimental value for water and neglecting $f(n)$, (the expression containing n in Equation 19), a is calculated to be 3.61 Å from the value of the slope, $105.0 \text{ cm}^{-1}/D^2$, (using the dipole values shown in Table 2). The value of a is not sensitive to the value of ϵ . This is because $f(\epsilon)$ reaches a plateau at $\epsilon \sim 10$. Larger values of ϵ do not significantly change $f(\epsilon)$. The SPC model results in values of $\epsilon=65 \pm 9^{140-143}$, thus falling well within the range where $f(\epsilon)$ is constant. The calculated value for a is in excellent agreement with the experimental value for the cavity size of crystalline indole, 3.6 Å. $f(n)$ was neglected in the calculation because this factor accounts for solvent electronic polarizability, which is not included in this model. Therefore, it does not contribute to the calculated $\Delta\bar{v}_f - \Delta\bar{v}_a$. If we were to fit the value

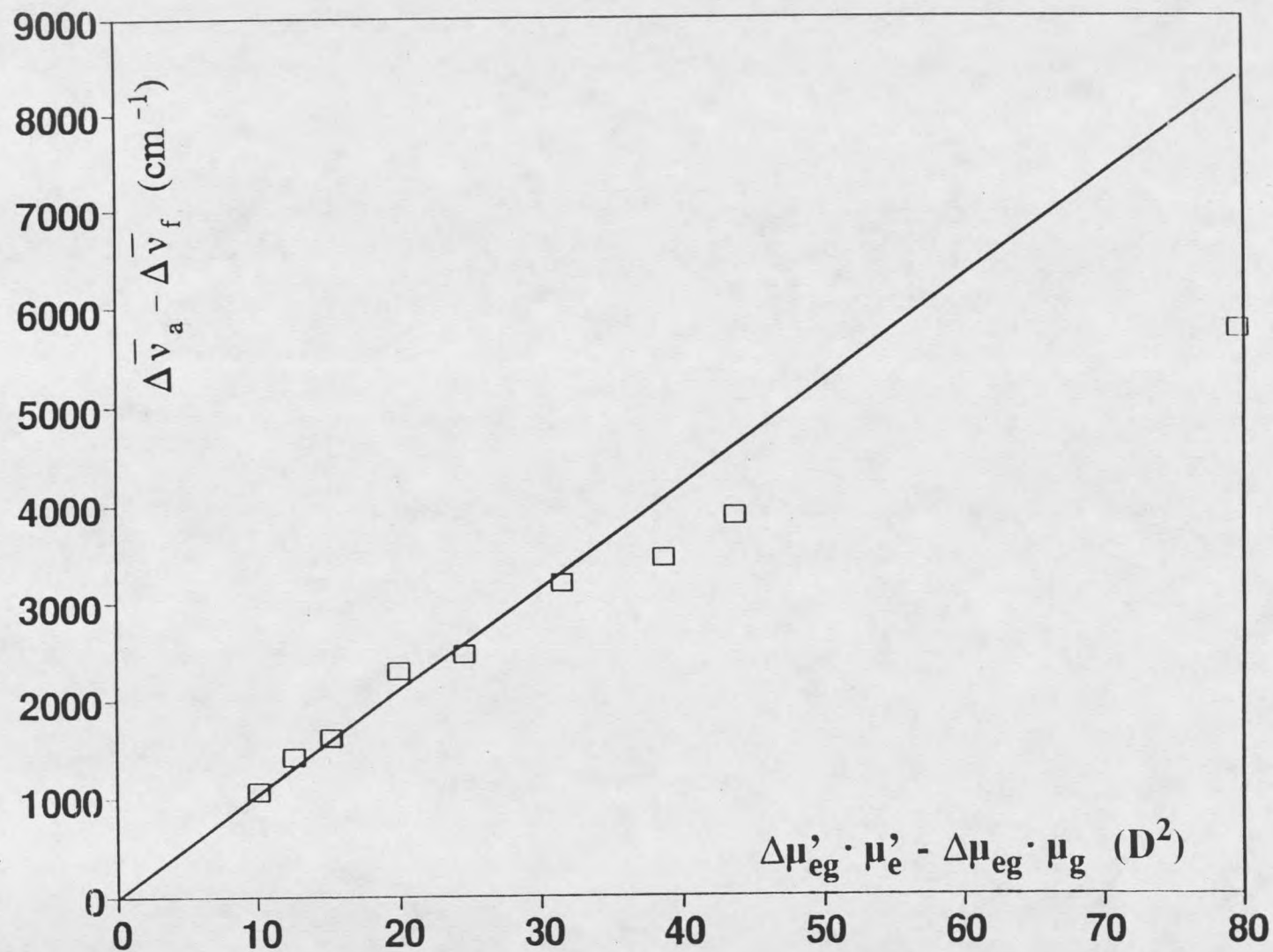


Figure 24. Dependence between absorption and fluorescence shifts and the permanent dipoles of ground and 1L_a states for indole in water.

Table 2. Calculated dipoles for indole and 3-methylindole for several geometries and environments.

		MD DIPOLE (Debye)				INDO/S DIPOLE (Debye)			
		S ₀ Geometry		¹ L _a Geometry		S ₀ Geometry		¹ L _a Geometry	
		S ₀	¹ L _a	S ₀	¹ L _a	S ₀	¹ L _a	S ₀	¹ L _a
Indole	vacuum	1.49	5.52	1.69	5.53	1.49	5.52	1.69	5.53
	Non-pol	2.28	6.53	2.32	6.59	2.52	6.49	3.96	8.47
	Polariz.	2.78	7.04	6.01	13.03	2.78	7.04	6.01	13.03
3MI	vacuum	1.21	6.96	1.42	6.84	1.21	6.96	1.42	6.84
	Non-pol	1.22	6.85	1.23	6.83	2.07	7.87	3.78	10.93
	Polariz.	2.24	7.86	4.56	11.66	2.24	7.86	4.56	11.66

corresponding to the polarizable model, we would have to use $a=4.06 \text{ \AA}$ for the cavity radius. A similar fit of $\Delta\bar{\nu}_f - \Delta\bar{\nu}_a$ would require that $a=4.27 \text{ \AA}$ for 3MI. Alternatively, the lower than expected values for $\Delta\bar{\nu}_f - \Delta\bar{\nu}_a$ at high dipoles could be qualitatively understood in terms of dielectric saturation induced by large dipole values.

In calculating Equation 19, it has been assumed that the electronic polarizability of the solvent included in Equations 17 and 18 cancels out^{97,98}. However, for this cancellation to be complete, one has to assume that the ground and excited state dipoles responsible for the absorption red shift have to match those inducing the fluorescence shift. This is not generally true, since the solvent field is polarized differently in each case because of the different solvent-field-inducing dipole. Therefore, our calculations, (even when we use the *polarizable* model) have an intrinsic error due to the lack of solvent polarizability equal to

$$\frac{(\mu_e'^2 - \mu_g'^2) - (\mu_e^2 - \mu_g^2)}{a^3} \left[\frac{n^2 - 1}{2n^2 + 1} \right] \quad (36)$$

The primed dipole moments are the dipole moments of the solute after the solvent relaxation has taken place, while the non-primed ones correspond to the dipoles of the solute during, (or before), excitation.

Using the dipole values in Table 2 and assuming that $n=1.33$ and $a=4.06 \text{ \AA}$ for indole and $a=4.27 \text{ \AA}$ for 3MI, Equation 36 yields values of 1175 and 640 cm^{-1} respectively, representing errors of ~10-20% for $\Delta\bar{\nu}_f - \Delta\bar{\nu}_a$.

Absorption

Figure 25 shows the 1L_a and 1L_b transition energy fluctuations for indole in water, (*polarizable* model). It looks very similar to Figure 19, the only difference being that the 1L_a vertical transition energies never go below those of 1L_b at this particular geometry. From these data, the calculated absorption red shift are $-620 \pm 400 \text{ cm}^{-1}$ for 1L_a and $-100 \pm 40 \text{ cm}^{-1}$ for 1L_b which do not compare very well with the experimental values of -2350 and -135 cm^{-1} respectively¹³. It has been mentioned in the previous section that the solvent electronic polarizability would represent 10-20% of the dielectric contribution to $\Delta\bar{\nu}_f - \Delta\bar{\nu}_a$. Therefore, the question arises as to which percentage of the absorption shift would be caused by the not included electronic polarizability of the solvent. From Equation 16, the expression for this contribution is

$$-\frac{\mu_e^2 - \mu_g^2}{a^3} \left[\frac{n^2 - 1}{2n^2 + 1} \right] \quad (37)$$

Using $n=1.33$ and $a=4.06 \text{ \AA}$ and $a=4.27 \text{ \AA}$ for indole and 3MI respectively, Equation 37 yields -550 cm^{-1} for indole and -621 cm^{-1} for 3MI. Both values are comparable in magnitude to the calculated values, (-620 and -550 cm^{-1}). Thus, it is expected that our method is not going to yield good quantitative results for the absorption shifts because the solvent electronic polarizability is not included.

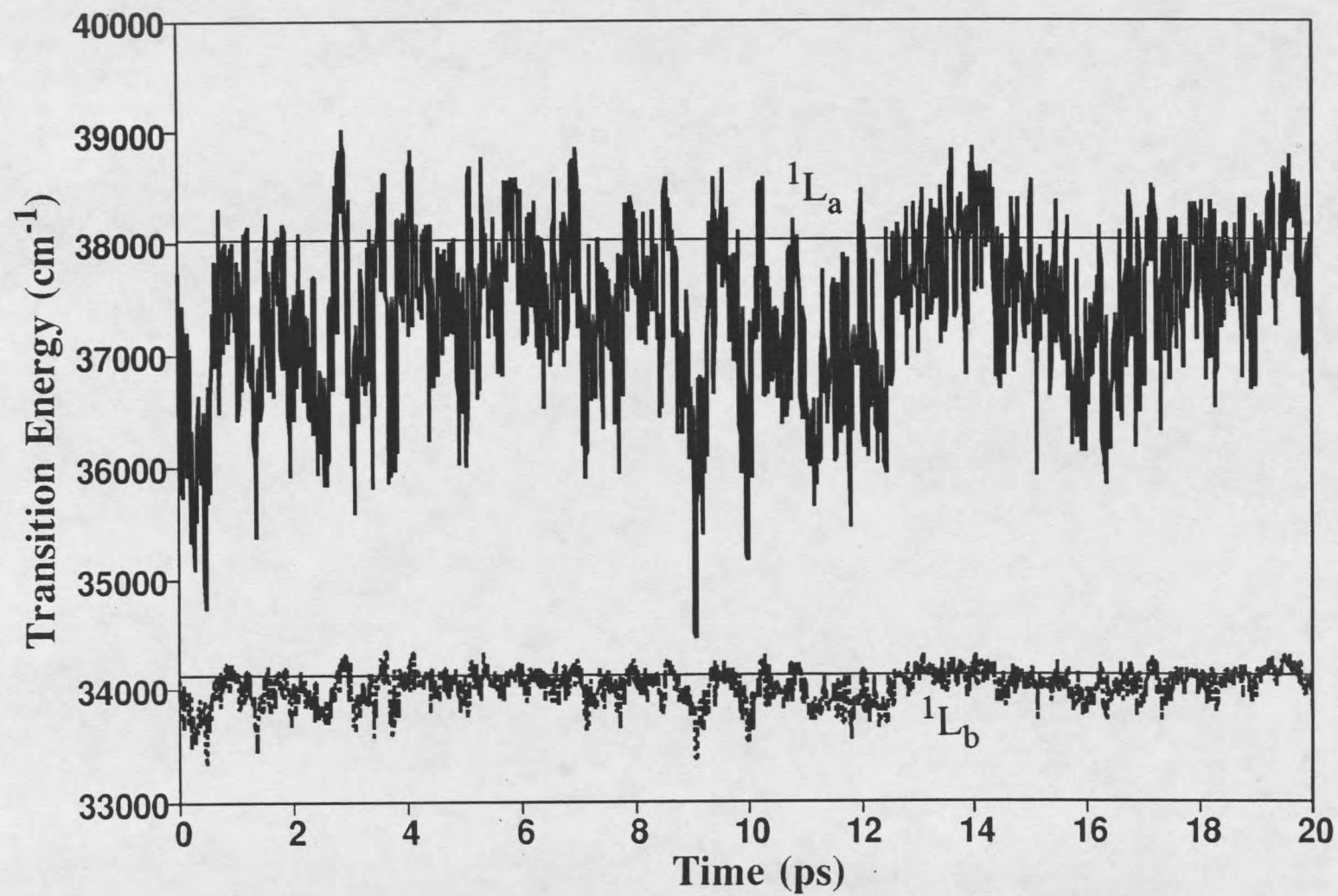


Figure 25. ¹L_a and ¹L_b transition energy fluctuations for indole in water.

3MI in Other Solvents

To further understand the influence of the solvent on the spectral shifts of indoles, we have done calculations on three other polar solvents: butanol, methanol, and dimethylether, (DME). All of the calculations reported here have been done using the polarizable model.

Fluorescence

The fluorescence shift for 3MI in these three solvents has been calculated by means of the direct response of the transition energies to the changing solvent field upon excitation.

The response of these solvents is slower than that of water, thus, it was necessary to follow the temporal evolution of the fluorescence for a longer period of time, (10 ps as opposed to 4). On the other hand, it was not necessary to sample the transition energies so often. As previously reported in the METHODS section, an INDO/S calculation was performed every 25 fs, except for the first 400 fs following excitation, when they were performed every 10 fs.

Figure 26 shows the evolution of the 1L_a and 1L_b transition energies following excitation for 3MI in butanol. Comparison to Figures 11 and 23 indicates that the solvent relaxation is much slower and induces a smaller red shift. Equivalent behavior has been found for 3MI in methanol and DME. These calculations are not shown because they are very similar to Figure 26. (See values in Table 3).

It was pointed out in page 57 that our calculated values are compared to the difference in the fluorescence maxima of a liquid¹³¹ and a rigid solution¹³². Our

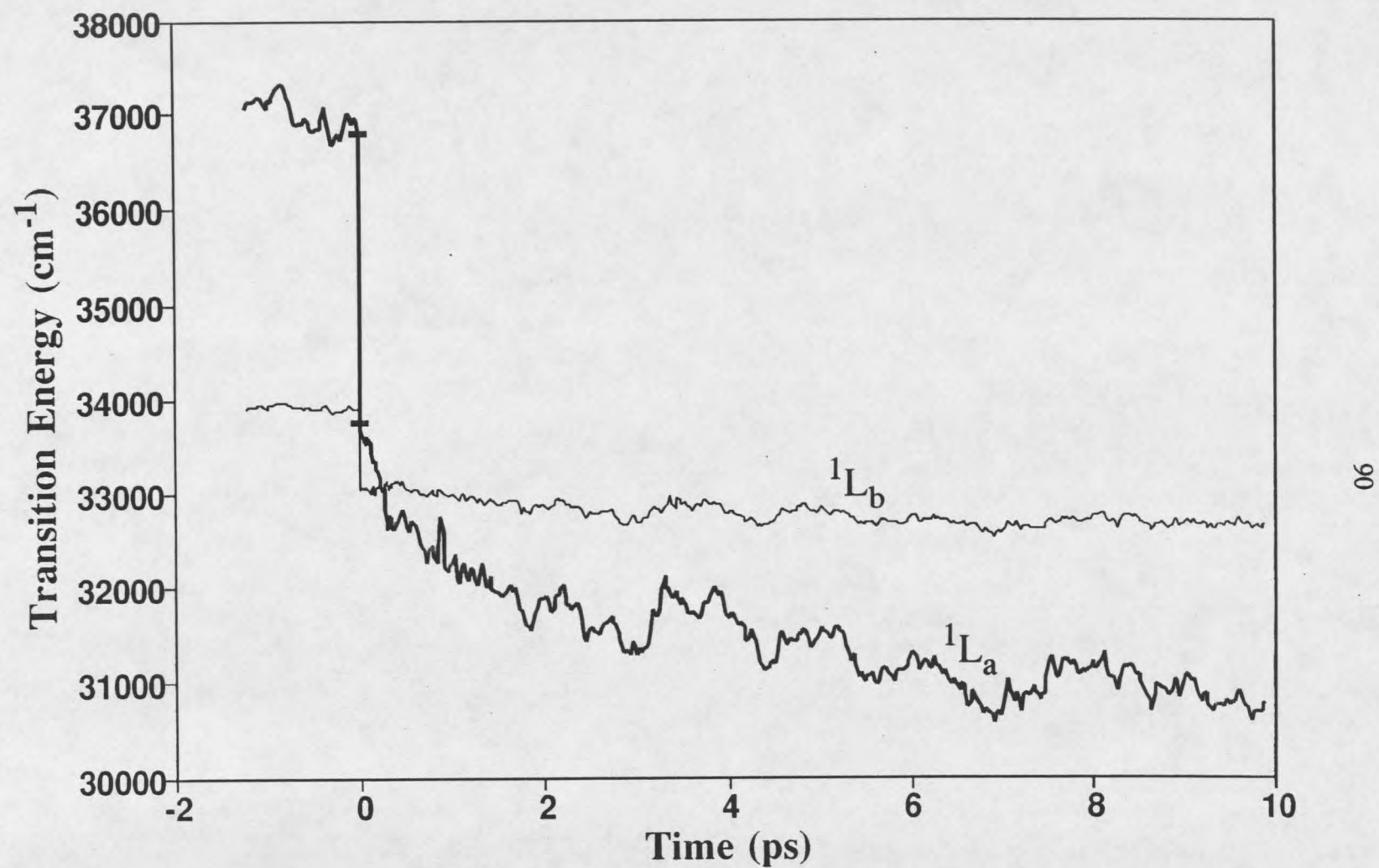


Figure 26. 1L_a and 1L_b fluorescence shifts for 3MI in butanol.

Table 3. Calculated and experimental absorption and fluorescence shifts.

		ABSORPTION SHIFT (cm ⁻¹)				FLUORESCENCE SHIFT (cm ⁻¹)			
		¹ L _b		¹ L _a		to vacuum ^a		to t=0 ^b	
		Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.
Indole+water	Semipol.	-60±50		-400±450		-2750±750		-2475±900	
	Pol.	-100±40	-135 ^c	-620±400	-2345 ^c	-6500±750	-4665 ^c	-5850±850	-3450 ^d
3MI+water	Semipol.	-80±50		-470±450		-3425±750		-2740±850	
	Pol.	-80±40	-95 ^c	-550±300	-1500 ^c	-5000±700	-6200 ^c	-4875±800	-3800 ^e
3MI+butanol		-45±50	-430 ^c	-200±350	-1635 ^c	-2650±800	-4300 ^c	-2530±800	-2700 ^f
3MI+methanol		-60±50	-360 ^c	-240±350	-1660 ^c	-2050±800	-4660 ^c	-2030±800	-2300 ^f
3MI+DME		+35±200	-430 ^{c,g}	+90±400	-1525 ^c	-1050±750	-2140 ^{c,g}	-1100±750	-

10

- a Fluorescence maxima relative to the maxima in vacuum, (these maxima correspond to the ¹L_b state). The fluorescence maxima in vacuum are calculated at the ¹L_a geometry.
- b Fluorescence maxima relative to the *rigid solvent* limit (see text).
- c From Ref. 13.
- d From Ref. 3.
- e From Refs. 131 and 132.
- f From Ref. 144.
- g Experimental values for 3MI in diethylether.

calculated value for butanol, $2530 \pm 800 \text{ cm}^{-1}$, compares fairly well with a similarly obtained experimental value¹⁴⁴ of 2700 cm^{-1} . Our calculated values agree with the experimental data in showing that there is a differential shift between water and butanol.

In the INTRODUCTION, it was noted that self consistent reaction field methods cannot predict the differential shift. Indeed, by inspection of Equation 18, it is easy to show that substitution of ϵ and n by 81 and 1.333 for water and 17.1 and 1.390 for butanol¹³ is not going to cause any differential shift unless the cavity radius changes significantly.

Table 3 lists the calculated red shifts and compares them to the experimental values, when available. The values obtained for the polar solvents are smaller than the experimental values, which is the opposite of what happened with 3MI in water. It must be noted, however, that at $t=10 \text{ ps}$, the solvent does not seem to be completely equilibrated to the excited state. This is apparent from the fact that the transition energies are still red shifting at the end of the simulation.

Table 4 contains the fits of the direct response to Gaussian and exponential decays for indole in H_2O , and for 3MI in the 5 solvents reported. The difference in temporal response are dramatic when comparing water to the other solvents. The Gaussian correlation time goes from 15-20 fs to 200-300 fs, while the exponential decays go from 200-400 fs to 5000-9000 fs. These numbers suggest, as mentioned in the previous paragraph, that solvent relaxation cannot be completed in only 10 ps.

Figure 27 shows the evolution of the $^1\text{L}_a$ transition energies for 3MI in H_2O , butanol, methanol, and DME for the first 50 fs after excitation. The transition energies were calculated every femtosecond, and the starting points were shifted in order to fit the

four trajectories into one graph. It is clear from this picture that the behavior is Gaussian for the 3MI+H₂O system. For the other solvents, there is not enough time to tell with absolute certainty, but the fact that the transition energies do not decay from $t=0$ is an indication that the decay is not exponential in nature.

Table 4. Fits for the direct response of the transition energy fluctuations to a Gaussian and an exponential decay, (including a constant term).

	c_G (cm ⁻¹)	τ_G (fs)	c_E (cm ⁻¹)	τ_E (fs)	K (cm ⁻¹)
Indole+H ₂ O	1460	15.3	4635	390	28700
3MI+H ₂ O	1680	15.7	3250	170	29050
3MI+D ₂ O	2115	25.1	3600	225	26550
3MI+butanol	1065	275	2075	5000	30530
3MI+methanol	1025	225	1275	5470	31290
3MI+DME	675	345	860	9230	32525

A better comparison of the actual time scale and red shift for these solvents is shown in Figure 28, which shows the temporal evolution of the transition energies for 3MI in the four solvents for a period of 10 ps. In this long time scale, the response in water seems almost instantaneous. The response in methanol and butanol look very similar, but the response in DME is much smaller and slower.

This agrees with the interpretation of Figure 13 given previously, which stated that the fast response is due to rotational reorientation of the hydroxyls following excitation to the ¹L_a state. Each water molecule has two of these hydrogens, while the alcohols have only one per molecule and DME has none. Furthermore, the smaller size of the water molecules allows the presence of more of them closer to the solute molecule.

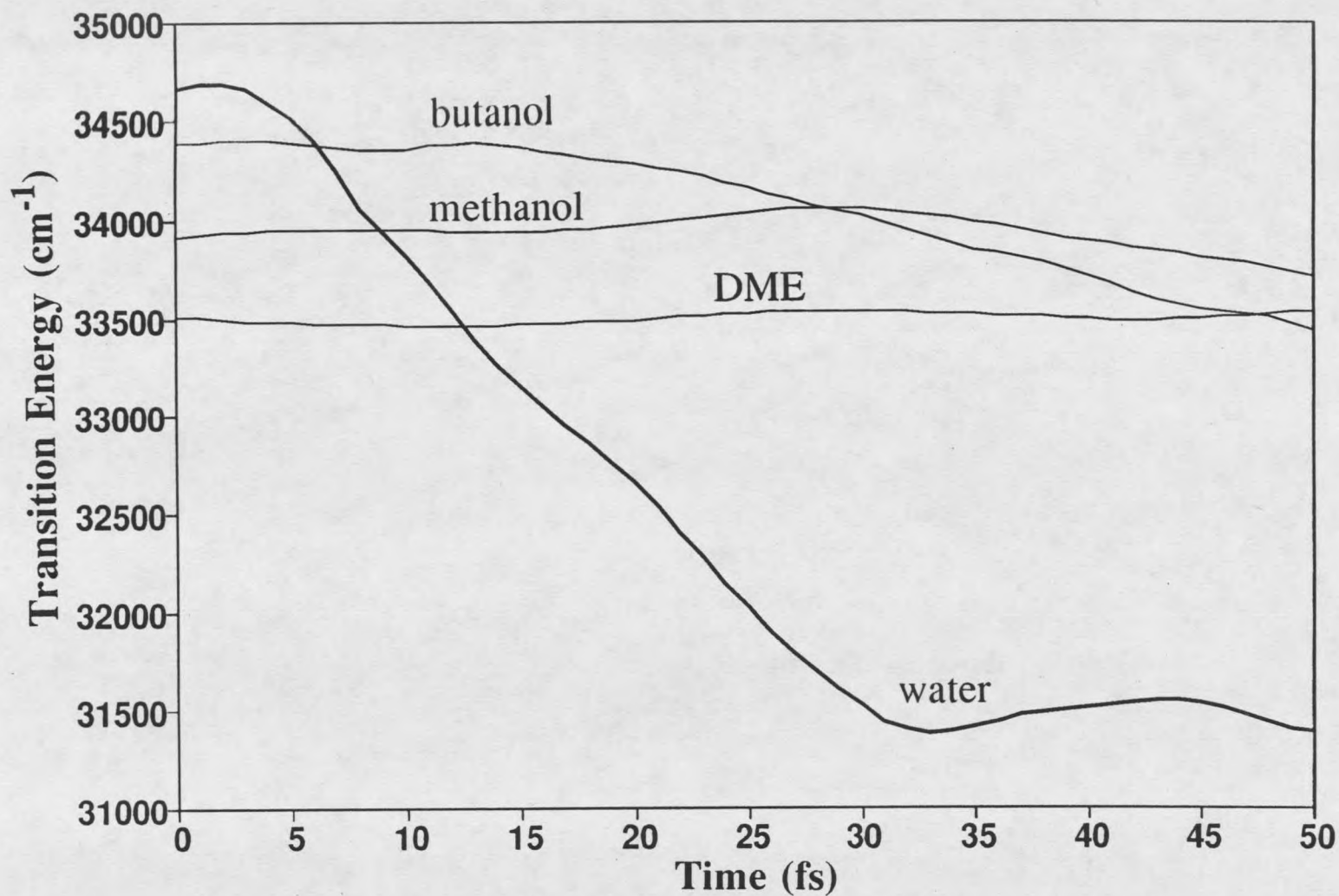


Figure 27. $^1\text{L}_a$ fluorescence shift for 3MI in water, butanol, methanol, and DME, for the first 50 fs after excitation.

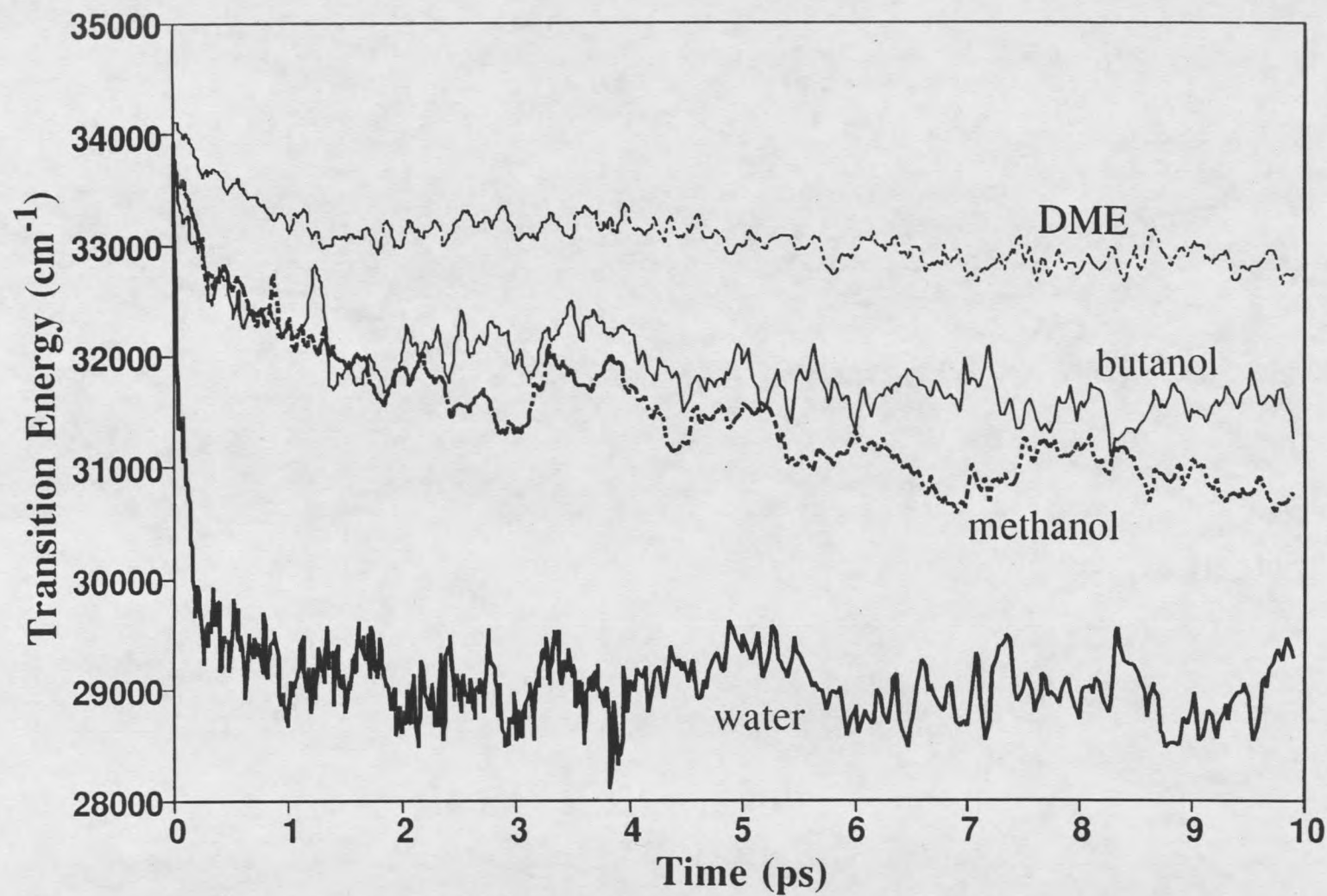


Figure 28. 1L_a fluorescence shift for 3MI in water, butanol, methanol, and DME, for the first 10 ps after excitation.

Figure 15 helped establishing that the hydroxyl induced red shift was not a matter of hydrogen bond formation initially, but rather an inertial phenomenon due to realignment of dipoles located at favorable positions. Thus, the larger the number of solvent molecules close to the solute, the higher the number of possible red shifting interactions, and the larger contribution to the red shift.

The slow, (exponential), decay also depends strongly on the solvent properties. For water, values for the longitudinal relaxation time have been reported ranging from 180 fs, (TIP4P model)¹⁴⁵, to 420 fs, (ST2 model)⁴⁵. These results are based on calculations of dielectric responses on continuum based models, and they agree well with our reported values for the correlation times for the exponential decay. In this context, the longitudinal relaxation time, (τ_L), can be viewed as a collective, (coupled), response of many solvent molecules to the changing field generated by the solute. The picture is not so clear for the other solvents. From experimental and calculated data reported by Fleming *et al.*¹⁴⁶, τ_L can be obtained for *n*-propanol at 253 K. This value for the longitudinal relaxation time, 1.93 ps, can be crudely compared to those calculated here for the correlation time of the exponential decay, (τ_E), in the five solvents studied here. It is larger than those reported for H₂O and D₂O, but smaller than our calculated values for τ_E in the other three solvents. Still, the emerging picture indicates that the slow response arises from the combined action of the solvent molecules trying to relax by slowly overcoming the action of their kinetic, (thermal), energy. Thus, the water response is much faster than those of the other solvents because of its larger dipole and smaller mass and size.

Absorption

Figure 29 shows the 1L_a and 1L_b transition energy fluctuations for 3MI in butanol. A comparison with Figure 19 indicates that the amplitude of the oscillations is much smaller in the case of butanol. Also, the graph is less "crowded" in this case, indicating that the solvent motion around the 3MI molecule is slower in butanol than it is in water. The behavior is very similar for 3MI in methanol and DME. Figure 30 shows the distribution of 1L_a and 1L_b transition energies for 3MI in the four solvents studied. Each graph is normalized differently, thus, there is no information to be obtained from the relative "intensities". However, it can be clearly seen that the linewidths are related to the polarity of the solvent, being largest for the water solution, (2000 ± 100 and 400 ± 50 cm^{-1} for 1L_a and 1L_b , (FWHM)), and narrowest for 3MI in DME, (650 ± 100 and 150 ± 50 cm^{-1} , respectively). The other systems show intermediate widths of 1400 ± 100 and 300 ± 50 cm^{-1} for 3MI+methanol and 1600 ± 100 and 300 ± 50 cm^{-1} for 3MI+butanol.

Calculated values for the absorption red shift for 3MI in butanol, methanol, and DME are shown in Table 3. Similarly to water solution, the results match poorly the experimental ones as a result of the lack of electronic polarizability of the solvent. The results are particularly poor for the DME solution, a case in which our calculations predict a blue shift instead of a red shift for the absorption. This is probably due to the fact that the solvent motion is very slow. Therefore, it can be locked in some particular conformation that causes the average blue shift. The time scale of the calculation does not allow for the solvent to reach a conformation more suitable to yield a red shift.

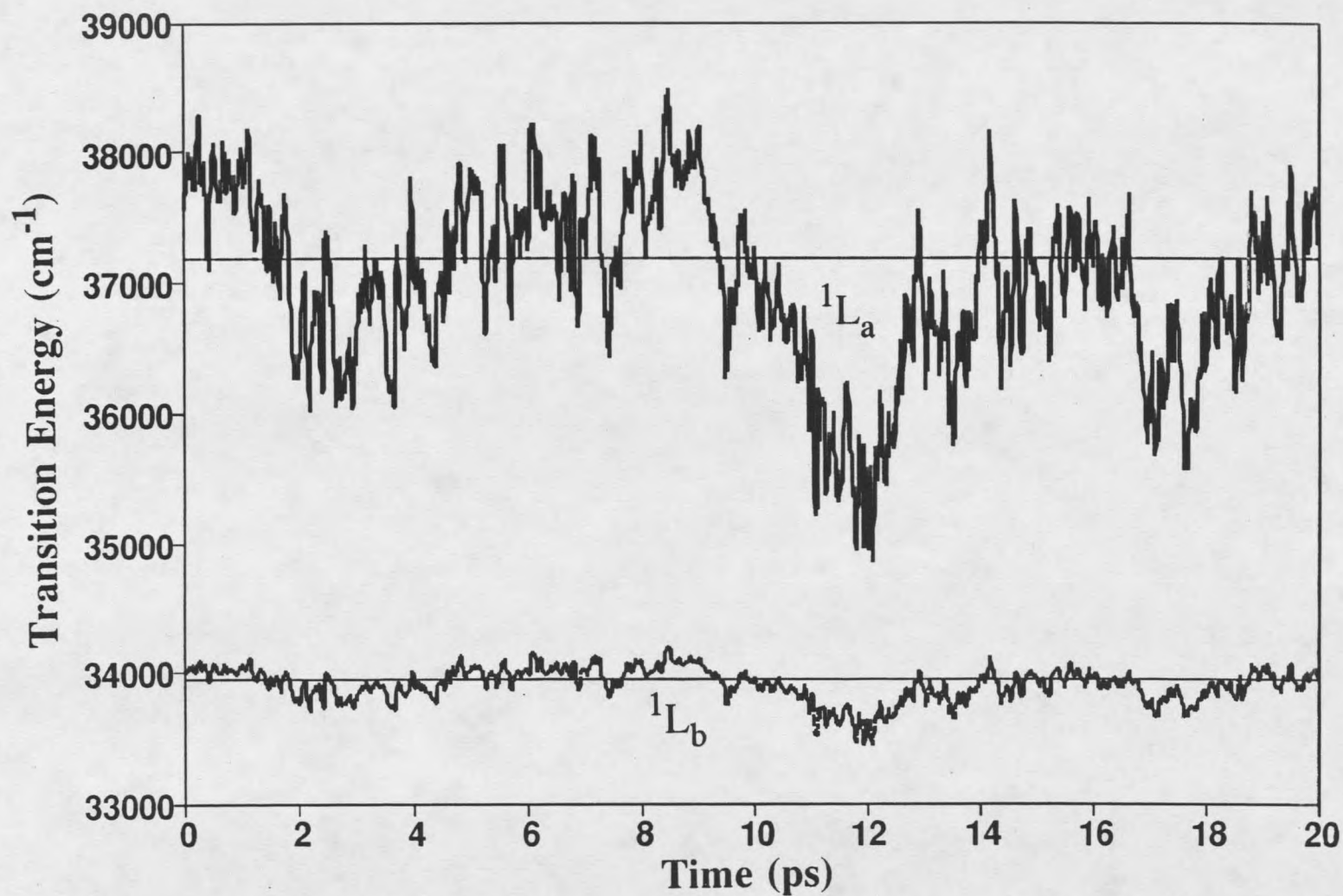


Figure 29. 1L_a and 1L_b transition energy fluctuations for 3MI in butanol.

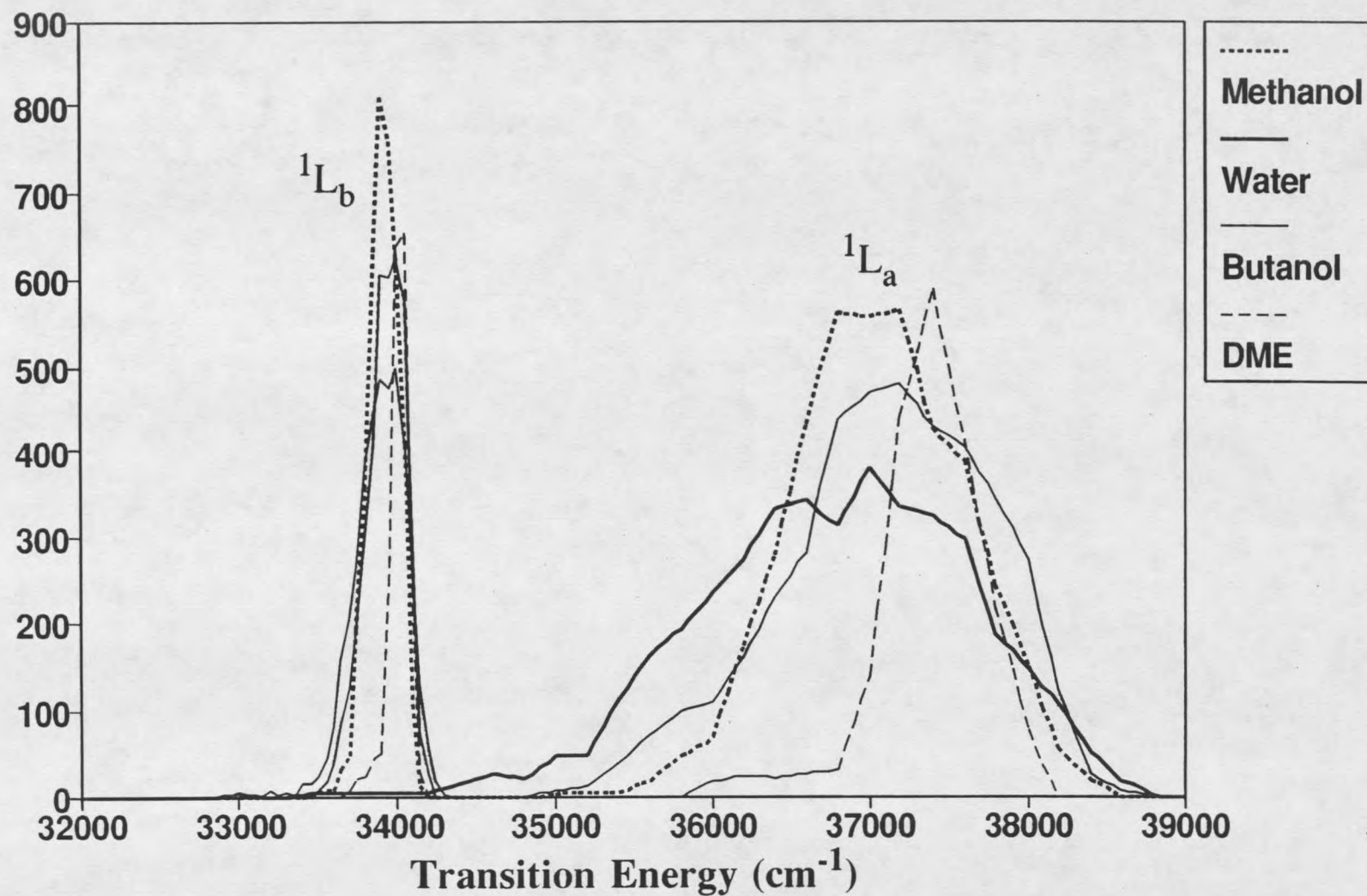


Figure 30. Distribution of transitions energies for 3MI in water, butanol, methanol, and DME.

Free Energies of Solvation

An estimate of the difference in free energy of solvation between ground and excited states can be obtained by using the calculated shifts according to Equation 21. This equation can also be used to obtain the corresponding "experimental" values from spectroscopic data.

For 3MI in water, a experimental ΔA_s can be calculated using the red shift values provided by Lami and Glasser¹³ for $\Delta \bar{\nu}_a$, (-1500 cm^{-1}), and $\Delta \bar{\nu}_f$, (-6200 cm^{-1}), (see Table 3). The result is $\Delta A_s = -11.0$ kcal/mol. Using our calculated values we get $\Delta A_s = -7.9$ kcal/mol. It is interesting to note that our calculations overestimate $\Delta \bar{\nu}_a - \Delta \bar{\nu}_f$, but they underestimate $\Delta \bar{\nu}_a + \Delta \bar{\nu}_f$, because the contributions from the electronic polarizability of the solvent do not cancel. We can also calculate ΔA_s using Equation 20 along with the values for μ_g , (2.24 D), and μ_e , (11.66 D), reported in Table 2, and using $a = 4.27$ Å, (calculated in 3MI in Water. Fluorescence). These values yield $\Delta A_s = -11.0$ kcal/mol, which is surprisingly close to the experimental value considering that this procedure is not very good in calculating the absorption red shift.

Similarly, for indole, an experimental value for the free energy of solvation is found to be -10.0 kcal/mol. From our calculated $\Delta \bar{\nu}_a$ and $\Delta \bar{\nu}_f$, we obtained $\Delta A_s = -10.2$ kcal/mol; and using Equation 20, with $\mu_g = 2.78$ D, $\mu_e = 13.03$ D, and with $a = 4.06$ Å, (see Indole in Water. Fluorescence), we got $\Delta A_s = -17.1$ kcal/mol.

Free energies of solvation can also be obtained for the other three solvents. From the absorption and fluorescence shifts reported by Lami and Glasser¹³, we can get values of ΔA_s equal to -8.5, -9.0, and -5.2 kcal/mol for butanol, methanol, and diethylether.

Using our calculated values for $\Delta\bar{v}_a$ and $\Delta\bar{v}_f$, we obtained values for the free energies of solvation of -4.1, -3.3, and -1.4 kcal/mol for butanol, methanol, and dimethylether, respectively.

Once again, the calculated $\Delta\bar{v}_a + \Delta\bar{v}_f$ underestimate the experimental value, except for the case of indole in water, possibly because the exaggerated calculated dipoles for indole may compensate for the lack of solvent electronic polarizability.

3MI in Butanol at 0 K

When comparing our calculated values for the fluorescence red shift of 3MI in water at room temperature, (see 3MI in Water), it was mentioned that the "experimental" values corresponded to a lower limit because even in the case where the solvent was presumed to be rigid, (like in a glass), the individual molecules could reorient as to position their dipoles in a more favorable position with respect with the new electric field generated by the solute, thus causing a red shift. This shift would be very fast and could be undetected by current experimental techniques.

To test the validity of this assumption, a series of calculations was performed on a system consisting of a 3MI molecule surrounded by 44 butanol molecules at 0 K. The method used in these calculations has been described in the METHODS section.

As it was expected that the ensemble would shrink when cooling, periodic boundary conditions were no longer considered to be convenient, since they can be interpreted as constant volume conditions. Therefore, we chose to use a spherical cluster for the 3MI+butanol system. Its total volume did decrease during the simulation.

Figure 31 shows a graph of the 1L_a transition energy for 3MI in butanol at 0 K

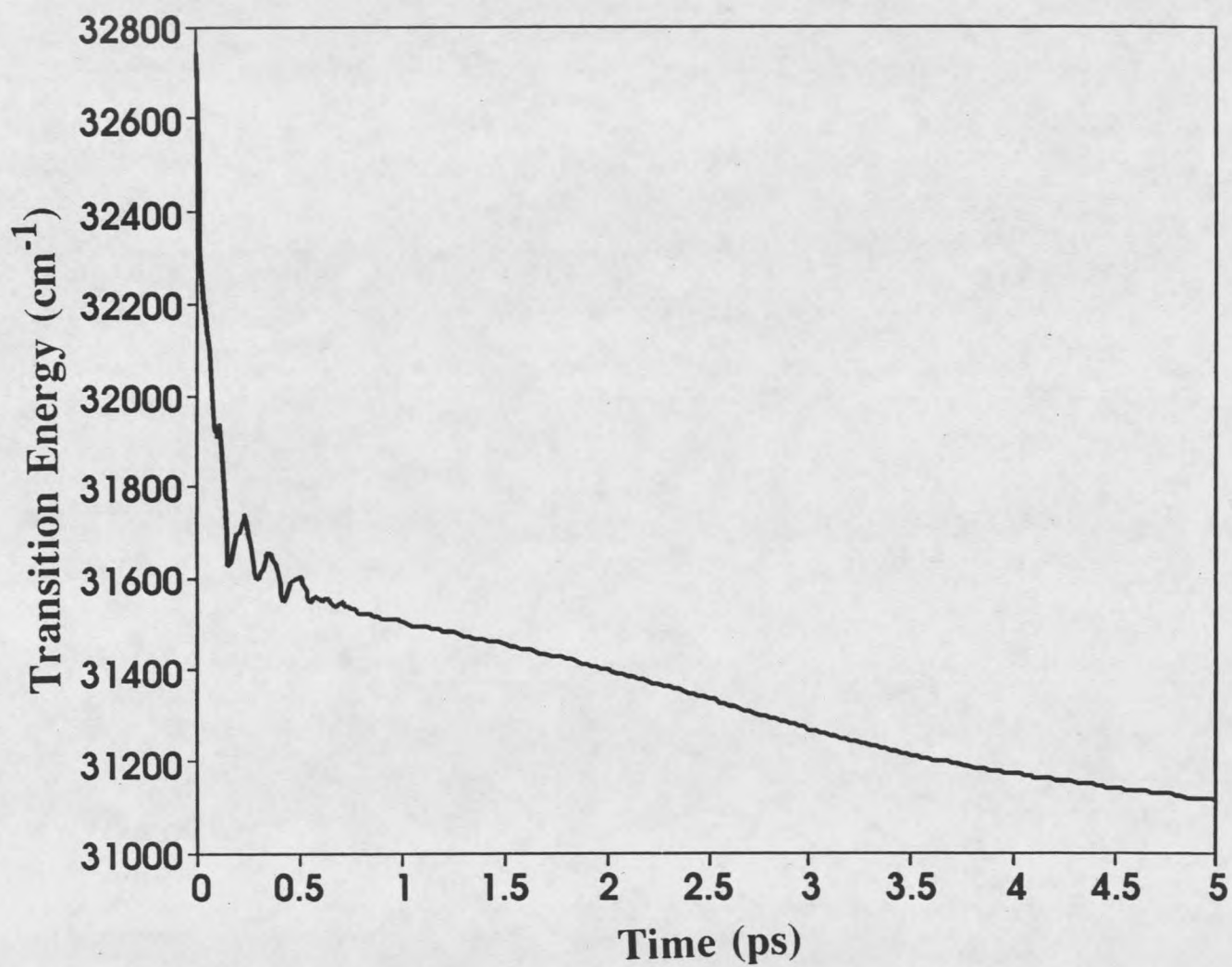


Figure 31. 1L_a fluorescence shift for 3MI in butanol at 0 K, (canonical ensemble).

for the first 5 ps after excitation. A decay of 1000 cm^{-1} takes place in the first 500 fs, indicating that even at 0 K there is enough solvent motion to induce a significant red shift. Visual inspection of the solvent motion shows that the red shift is caused by rotation of the hydroxyls in the solvent molecules in response to the new solute dipole. Their movement takes them back and forth past the "equilibrium" position, thus causing the ringing seen in the first 500 fs of the trajectory.

Molecular dynamics, by default, scales the velocities of the molecules in order to match their kinetic energy to that corresponding to the requested temperature of the system. In this respect, it behaves like a canonical ensemble, because its energy is slowly being dumped to a surrounding heat bath. This behavior, which is very convenient when working at room temperature, is somehow artificial at 0 K, since it is hard to estimate how fast the energy absorbed by the molecule will dissipate.

To study the other limit, (where the energy acquired by the molecule is not dumped), a set of simulations was run where the total energy of the system was kept constant, effectively resembling a microcanonical ensemble. The starting points of these calculations were the same used with the canonical ensemble simulations, in order to be able to compare them.

Figure 32 shows a graph of the decay of the 1L_a transition energy using the microcanonical ensemble and starting at the same point as in Figure 31. The first 200-300 fs of both trajectories are very similar, (note the difference in scale in the two graphs), both in terms of red shift and in terms of molecular motion. However, contrary to what happened in Figure 31, the solvent energy was not being dumped, but rather, it

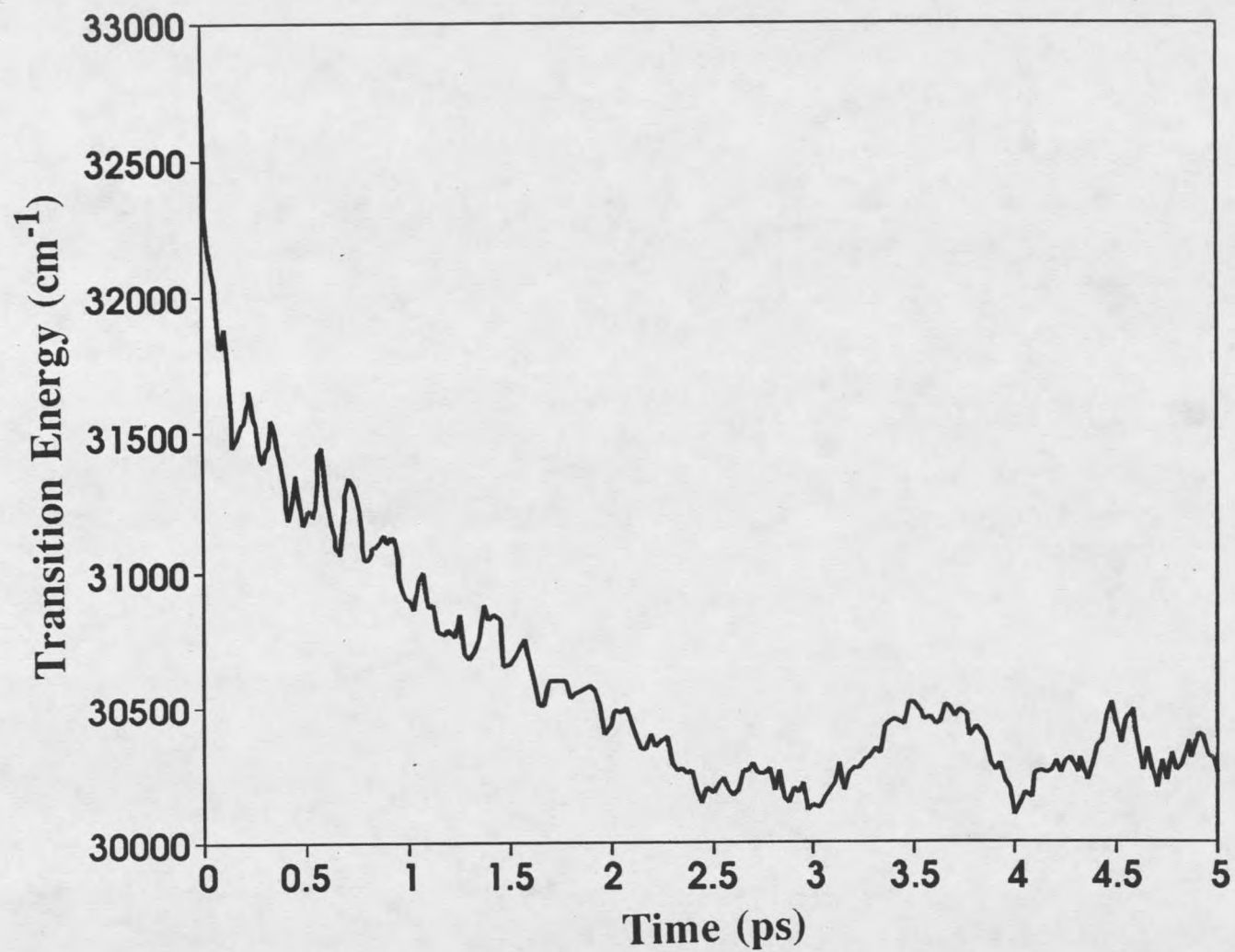


Figure 32. 1L_a fluorescence shift for 3MI in butanol at 0 K, (microcanonical ensemble).

was transmitted to the other molecular degrees of freedom. After $t=0.5$ ps, other vibrations were visible in the solvent molecules and soon after that, part of this energy was transformed into translational kinetic energy, which resulted in a larger red shift, (2500 cm^{-1} at $t=5$ ps vs 1500 cm^{-1} in Figure 31). At $t=5$ ps, the temperature of the system was found to be ~ 4 K.

Analyses of other trajectories showed similar results to these reported here, with minor differences in the shape and magnitude of the decay.

Although neither of these models is quite realistic, they represent limiting cases to what can be expected to happen when a molecule in a rigid solvent is excited. Solvent reorganization can take place very fast, producing a non negligible red shift. Thus, the experimental results reported in Table 3 may be, as mentioned before, $1000\text{-}2000\text{ cm}^{-1}$ too small, if these simulations are realistic.

Rotational Conformers of Tryptophan in Vacuum

The side chain of tryptophan is a flexible structure with several degrees of freedom. At room temperature, it can adopt several conformations. Each of those will have distinctive 1L_a and 1L_b origins resulting in a more complicated spectrum with respect to those of indole or 3MI. Using molecular mechanics, we found 62 different conformers for the neutral, (non-zwitterionic), species in vacuum, corresponding to local minima of the potential energy surface. However, the population of each conformer is different, depending on the relative energy of each minimum. Table 5 shows a list of the 62 conformers, the angles defining the orientation of the side chain, their energy in kcal/mol, (relative to the absolute minimum, which corresponds to conformer # 46), the 1L_a and

Table 5. List of conformers of tryptophan at 300 K.

#	Angles ^a					Energy ^b	¹ L _b ^c	¹ L _a ^c	% ^d
	#1	#2	#3	#4	#5				
1	-78	161	123	3	-45	4.4540	34095	37961	0.01
2	-79	163	63	178	-61	2.2283	34091	37916	0.46
3	-91	-64	94	5	-43	4.0161	34015	37476	0.02
4	-86	-63	79	179	-66	3.2672	34027	37560	0.08
5	-94	67	90	10	-37	4.6759	34099	37943	0.00
6	-98	64	55	179	-63	0.6979	34045	37633	5.96
7	-90	-66	-115	-178	-67	4.0394	33999	37362	0.02
8	-95	68	-125	180	-64	0.0181	34071	37925	18.63
9	-104	74	176	-10	-61	5.1108	34096	38088	0.00
10	82	74	174	-10	-61	4.3671	33965	37097	0.01
11	81	67	-135	180	-64	0.3381	34008	37352	10.89
12	92	-61	-102	-179	-69	3.8205	34094	37949	0.03
13	86	-65	96	5	-45	3.6432	34131	38260	0.04
14	91	-61	80	-179	-68	2.5182	34082	37927	0.28
15	82	70	101	8	-40	4.6601	34024	37378	0.01
16	78	64	54	179	-63	1.0162	34067	37672	3.49
17	98	163	125	2	-46	4.8047	34011	37468	0.01
18	96	164	62	178	-61	2.4873	34026	37568	0.30
19	96	166	-133	-178	-62	2.3552	34032	37645	0.37
20	-80	163	58	178	57	2.5044	34075	37804	0.29
21	-78	163	88	7	54	3.3438	34026	37568	0.07
22	-87	-62	82	2	54	3.1417	34038	37570	0.09
23	-84	-60	77	180	56	4.5369	34048	37637	0.01

Table 5-Continued

#	Angles ^a					Energy ^b	¹ L _b ^c	¹ L _a ^c	% ^d
	#1	#2	#3	#4	#5				
24	-93	67	76	8	57	2.9070	34100	37966	0.15
25	-99	64	51	179	58	0.9495	34052	37690	3.90
26	-88	-69	-160	-178	54	5.5549	33978	37246	0.00
27	-95	68	-130	179	59	0.8910	34078	37992	4.93
28	-99	72	-151	-4	57	7.5881	34103	38119	0.00
29	84	71	-167	-6	53	7.0546	33971	37139	0.00
30	82	67	-144	180	58	0.9949	33995	37320	3.62
31	94	-58	-86	180	60	5.2631	34065	37732	0.00
32	89	-63	83	2	54	2.5681	34117	38133	0.26
33	93	-60	78	-179	56	3.5767	34064	37787	0.05
34	83	68	78	8	56	3.0579	34055	37562	0.11
35	78	64	48	179	58	1.2213	34066	37674	2.48
36	96	164	87	7	53	3.6413	34030	37560	0.04
37	94	163	57	178	56	2.7609	34037	37631	0.19
38	94	164	-145	-178	56	2.9606	34037	37679	0.13
39	-82	161	61	179	179	2.8603	34073	37791	0.16
40	-81	160	78	5	-177	6.6820	34101	37954	0.00
41	-85	-63	73	-2	180	4.3119	34037	37599	0.01
42	-85	-62	79	180	179	3.2103	34039	37626	0.09
43	-94	66	65	4	178	5.1973	34087	37852	0.00
44	-99	64	54	180	178	0.5867	34045	37664	7.18
45	-89	-63	-102	180	-178	4.0166	34029	37529	0.02

Table 5-Continued

#	Angles ^a					Energy ^b	¹ L _b ^c	¹ L _a ^c	% ^d
	#1	#2	#3	#4	#5				
46	-94	68	-123	179	180	0.0000	34073	37955	19.20
47	-94	69	-84	8	-179	6.0125	34097	38059	0.00
48	84	70	-156	-3	-174	6.1222	33988	37254	0.00
49	80	67	-134	180	180	0.3505	34017	37428	10.67
50	91	-60	-87	180	180	3.6351	34076	37814	0.04
51	92	-64	72	-3	178	3.9198	34106	38097	0.03
52	91	-61	81	-178	178	2.4757	34070	37834	0.30
53	81	66	64	4	178	5.3741	34084	37748	0.00
54	78	64	51	180	178	0.8945	34074	37741	4.28
55	95	162	-165	-5	-172	8.7091	34039	37625	0.00
56	95	162	61	179	179	3.3432	34041	37629	0.07
57	95	163	-132	-179	-179	3.3030	34048	37705	0.08
58	96	162	77	5	-177	7.1892	34039	37566	0.00
59	-79	165	-132	-178	-61	2.1155	34056	37686	0.55
60	-81	164	-144	-178	57	2.0648	34037	37584	0.20
61	-83	163	-131	-179	-179	2.8274	34037	37571	0.17
62	-84	161	-165	-5	-173	8.1868	34043	37602	0.00

- a The angles, in degrees, are defined as: #1: C δ 2 (C9 in indole)-C γ (C3)-C β -C α ; #2: C γ -C β -C α -C O ; #3: C β -C α -C O -O H ; #4: C α -C O -O H -H; and #5: C β -C α -N-H1.
- b Energy of the conformer, in kcal/mol, relative to the absolute minimum, (#46).
- c Transition Energy, in cm⁻¹.
- d Relative percentage of the conformer, at 300 K. Those labelled "0.00" correspond to a conformer with a relative population of less than 0.01%.

1L_b transition energies, (in cm^{-1}), and their relative population at 300 K. The angles are defined as follows: #1: $C\delta 2-C\gamma-C\beta-C\alpha$, ($C\delta 2$ is $C9$ and $C\gamma$ is $C3$, see Figure 6, $C\beta$ is the carbon attached to $C3$, and $C\alpha$ is bonded to $C\beta$ and having $-\text{NH}_2$ and $-\text{COOH}$ as substituents); #2: $C\gamma-C\beta-C\alpha-C_O$, (C_O is the carboxylic carbon); #3: $C\beta-C\alpha-C_O-O_H$, (O_H is the oxygen in the $-\text{OH}$ group); #4: $C\alpha-C_O-O_H-H$; and #5: $C\beta-C\alpha-N-H1$.

From Table 5, we can see that, among the 62 conformers, only 12 of them contribute significantly, ($>1\%$), to the total population. These 12 conformers account for $\sim 95\%$ of the tryptophan molecules, (non-zwitterionic), in tryptophan vapor at 300 K.

Figure 33 shows the simulated spectrum, (for the 1L_a region), for the 12 most populated conformers of tryptophan, (weighted by their relative population factor). It can be understood from this figure that the spectrum of tryptophan in solution has to be very crowded, considering that to this broadness, (as a result of the different conformers), one has to add the inhomogeneous broadening and Franck-Condon factors that each of these origins is going to have built upon.

Figure 34 shows the simulated spectrum for the 1L_b region of the 12 conformers, (note the different x-axis scale with respect to Figure 33). The relative ordering is similar for the two regions, (1L_a : #30<11<49<6<44<25<35<16<8<46<54<27; 1L_b : #30<11<49<6<44<16<35<25<54<8<46<27), but not identical. In particular, conformers # 16, 25, and 35; and # 8, 46, and 54 change relative positions when going from the 1L_a to the 1L_b transition energies.

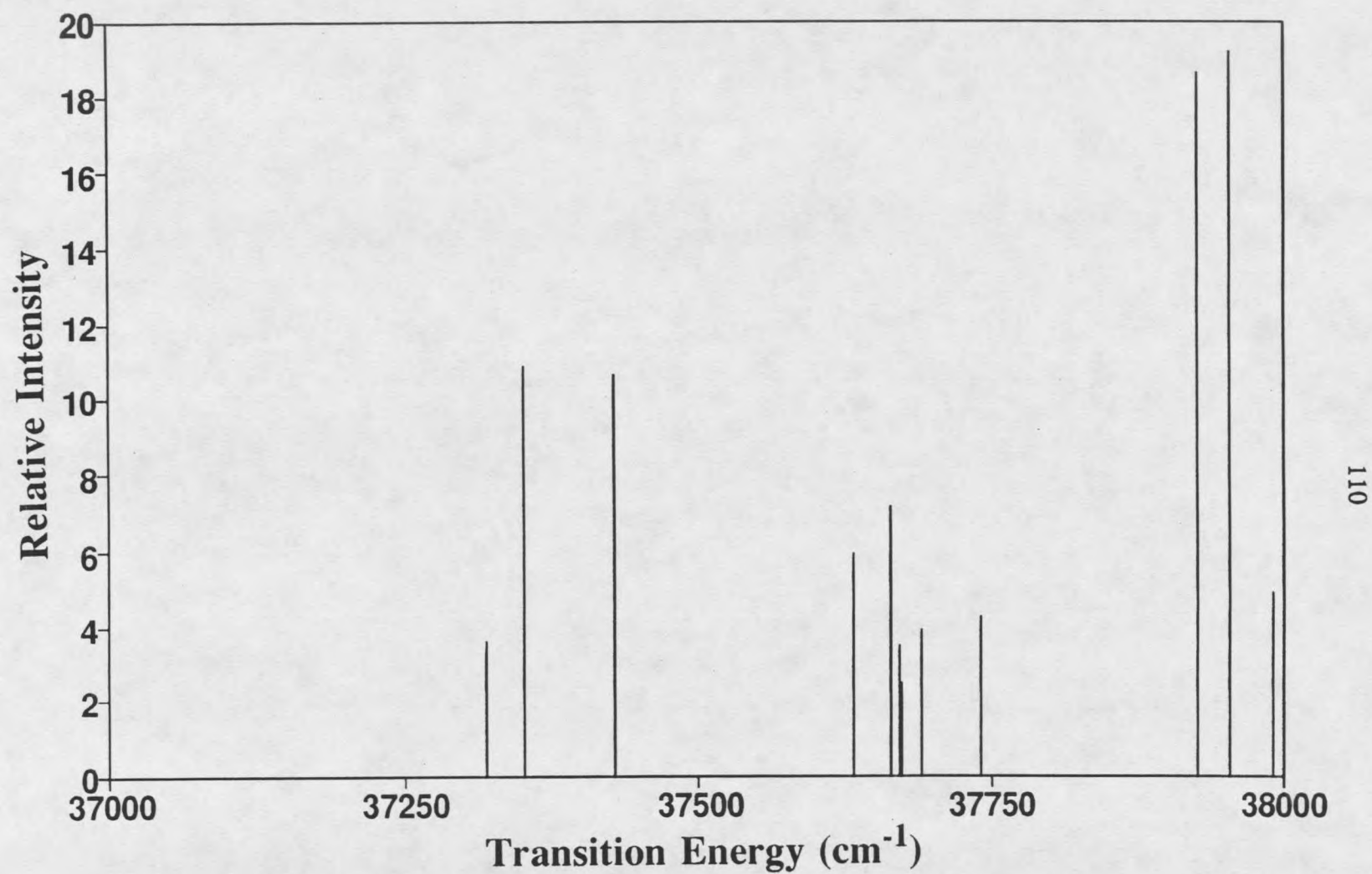


Figure 33. 1L_a transition energies, (weighted by their Boltzmann factors), for the 12 most populated conformers of tryptophan in vacuum at 300 K.

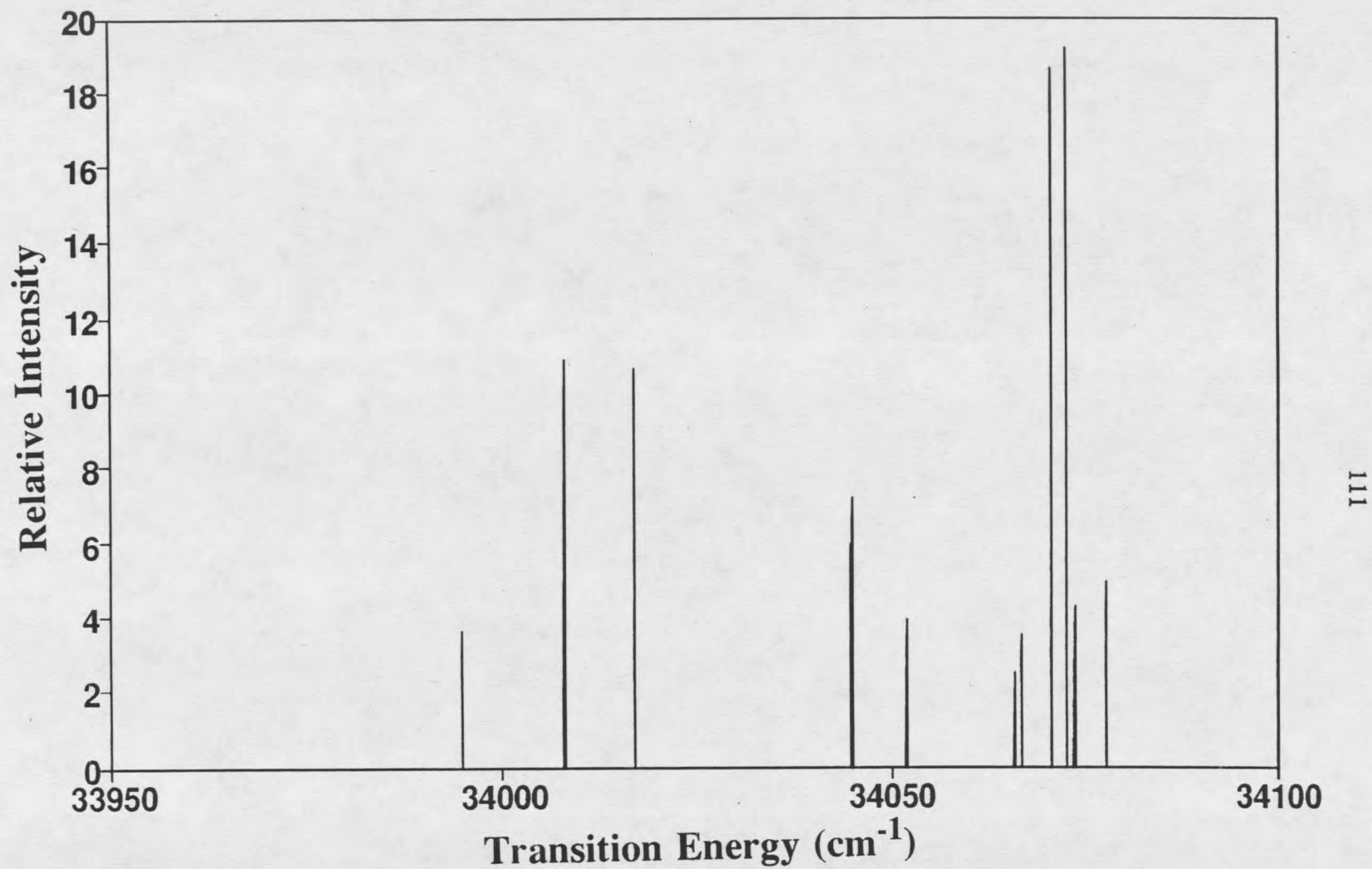


Figure 34. 1L_b transition energies, (weighted by their Boltzmann factors), for the 12 most populated conformers of tryptophan in vacuum at 300 K.

Careful inspection of the dihedral angle values reveals that the rotations around the $C\gamma-C\beta$, C_O-O_H , and $C\alpha-N$ bonds correspond to "two-fold axis", in the sense that two local minima can be found in a 360° rotation. This seems obvious in the last two cases, but it is less clear for the first, since one would expect three local minima per revolution around a carbon-carbon bond. It appears, however, that only those conformations with the bulky $-CH(NH_2)-COOH$ substituent roughly perpendicular to the ring, (either above or below), are stable, thus giving the "two-fold axis". Rotations around $C\beta-C\alpha$ or $C\alpha-C_O$ are "three-fold axis", in the sense that three local minima can be found every 360° . The latter is also surprising, but arises from the relative orientation of the $-COOH$ group with respect to the other two substituents of the $C\alpha$ carbon: $-H$ and $-NH_2$.

Considering the number of minima per 360° rotation around each bond, a total of $2 \times 3 \times 3 \times 2 \times 2 = 72$ conformers would be expected. These 72 were analyzed, but 10 of them did not correspond to a local minimum and went "downhill" along the potential energy surface, to end up in one of the 62 conformers presented in Table 5.

These calculations are relevant for the prediction of the behavior of tryptophan in supersonic jet. Levy *et al.*¹⁴⁷ have found six distinctive electronic origins in the spectrum of tryptophan in jet, each of them having different intensities and corresponding to a different conformer. A similar behavior was found for other tryptophan derivatives¹⁴⁸. Each of the origins has its own vibrational modes, and this will result in a more complicated spectrum that one can expect for indole, because there will be vibronic lines from each of the conformers present. This is why calculations of the type presented here can be an useful tool in order to help understanding the spectrum of tryptophan.

Experimental

Figure 35 shows a detailed one-photon excitation spectrum of indole in supersonic jet. Table 6 lists the frequencies for the observed transitions, along with their intensities relative to the transition at 717.8 cm^{-1} from the 1L_b origin and the assignments of some of the transitions^{27,35,127,149}. In this spectrum, the 1L_b origin, (at 0 cm^{-1}), is saturated, (we will use, in this section, the word *saturated* to indicate PMT saturation. This means that, after the fluorescence collected reaches a certain intensity level, the PMT sends a constant voltage to the board which integrates the signal). This is the reason why the intensities shown in Table 6 refer to the second highest peak in the spectrum. The ratio between the intensities of $^1L_b\text{ }0-0$ and $^1L_b\text{ }26_0^1$ reported in the table has been obtained from another spectrum. The spectrum shown in Figure 35 is very similar to that reported by Sammeth¹²⁷, but is colder, and no hot bands have been assigned.

All the transitions listed in Table 6 correspond to the spectrum of bare indole, except for the one at 19.1 cm^{-1} , which we believe corresponds to an intermolecular vibrational mode of the complex indole+He, as evidenced by the fact that its relative intensity with respect to other peaks changes with the backing pressure. In other words, if the backing pressure increases, (thus, increasing the ratio He/indole), the intensity of the peak goes up, because more complexes are formed. On the other hand, its relative intensity with respect to the origin of the indole+He complex is relatively constant, ($\sim 7-9\%$).

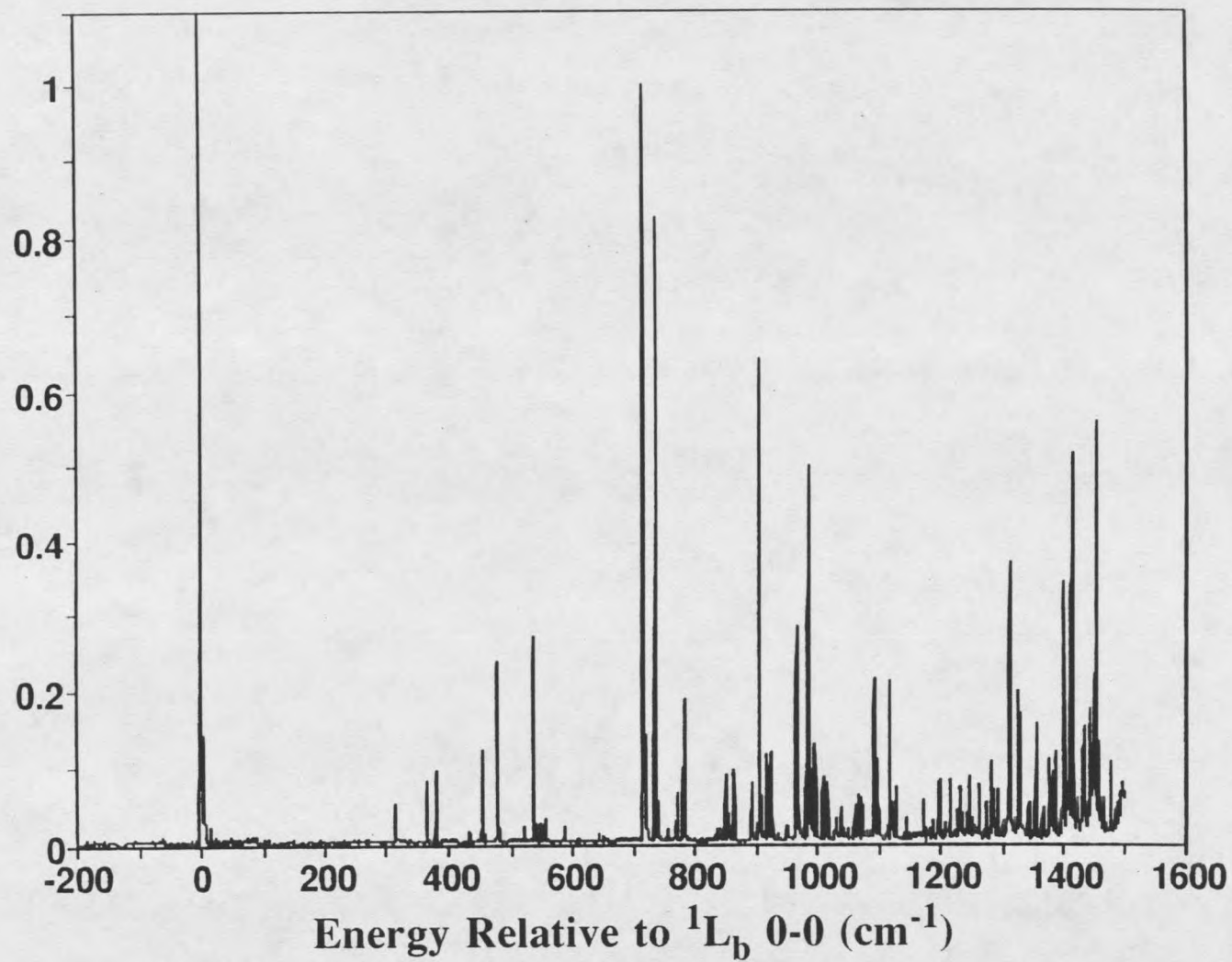


Figure 35. One-photon excitation spectrum of indole in supersonic jet.

Table 6. List of transitions in the spectrum of indole, along with their assignments.

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b	Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
0.0	3.74 ^c	¹ L _b 0-0	866.4	0.10	
19.1	0.02	Ind.+He (v ₁)	894.1	0.08	
316.6	0.05	42 ₀ ²	901.6	0.03	
365.7	0.08	41 ₀ ²	907.5	0.64	mix. v ₂₁ -v ₂₃
380.5	0.10	29 ₀ ¹	919.2	0.12	
436.1	0.01	39 ₀ ¹ 42 ₀ ¹	922.5	0.10	
454.9	0.12	¹ L _a 0-0	926.7	0.06	
461.2	0.01	39 ₀ ¹ 41 ₀ ¹	929.4	0.12	
480.4	0.24	¹ L _a 0-0	952.2	0.02	
524.1	0.02	37 ₀ ¹ 42 ₀ ¹	955.8	0.02	
539.8	0.27	27 ₀ ¹	968.2	0.29	mix. a" states
548.7	0.03	37 ₀ ¹ 41 ₀ ¹	972.5	0.06	
558.0	0.03	39 ₀ ²	984.3	0.14	
588.1	0.02		988.9	0.50	
645.0	0.01		994.5	0.10	36 ₀ ²
654.0	0.01		999.4	0.13	
717.8	1.00	26 ₀ ¹	1011.2	0.09	
720.1	0.47	29 ₀ ¹ 41 ₀ ¹ 42 ₀ ¹	1019.8	0.08	
736.6	0.83	37 ₀ ²	1032.3	0.04	
759.2	0.02		1039.2	0.05	
773.1	0.07	36 ₀ ¹ 39 ₀ ¹	1051.3	0.02	
783.8	0.19	¹ L _a (?)	1063.2	0.05	
837.0	0.02		1066.5	0.06	
841.6	0.02		1073.7	0.06	
851.1	0.07		1077.7	0.05	
854.7	0.09		1094.5	0.21	

Table 6-Continued

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b	Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
1100.4	0.11	¹ L _a	1310.5	0.10	¹ L _a
1115.6	0.02		1313.5	0.37	
1120.3	0.21		1322.5	0.03	
1127.5	0.04		1326.9	0.20	
1130.5	0.07		1327.9	0.18	
1146.4	0.04		1329.2	0.17	
1174.2	0.06		1345.2	0.05	
1179.8	0.02		1356.6	0.15	
1185.1	0.02		1368.3	0.05	
1189.7	0.03		1378.1	0.11	
1197.0	0.08		1380.4	0.07	
1199.4	0.08		1383.4	0.09	
1209.3	0.03		1387.1	0.08	
1215.3	0.08		1391.5	0.12	
1229.6	0.04		1400.2	0.09	
1232.9	0.07		1403.2	0.34	
1234.9	0.04		1409.9	0.08	
1244.2	0.04		1412.6	0.34	
1248.9	0.09		1415.3	0.52	
1263.2	0.08		1418.6	0.14	
1276.2	0.05		1425.3	0.06	¹ L _a
1283.8	0.11		1435.4	0.15	
1286.8	0.07		1439.1	0.05	
1295.2	0.07		1443.2	0.07	
1302.5	0.03		1445.8	0.17	

Table 6-Continued

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b	Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
1448.2	0.12		1463.7	0.06	
1449.5	0.12		1467.0	0.06	
1452.2	0.22	¹ L _a	1479.8	0.11	
1455.9	0.56	¹ L _a			

a Intensity relative to the 26₀¹ band.

b The assignments have been made with the aid of references 27, 35, and 127.

c This line is saturated in the spectrum in Figure 35. Its relative intensity with respect to the 26₀¹ band has been obtained from another spectrum.

The assignment of the ¹L_a transitions in Table 6 is based on the work of Callis *et al.*^{35,127}. We have performed several two-photon experiments on selected transitions, (¹L_b 0-0, ¹L_b 26₀¹, ¹L_a (at 480.4 cm⁻¹), ¹L_a (at 1455.9 cm⁻¹), and the peak at 783.8 cm⁻¹), and the Ω values we measured agree with theirs, in spite of the spatial differences in the fluorescence collection: while they collect all the light in a surface larger than one half of an imaginary sphere centered at the intersection of laser and molecular beams¹²⁷, we collect the fluorescence projected towards two regions defined by the intersection of such a sphere with a cylinder of radius 50 mm perpendicular to the plane containing the laser and molecular beams, (see Figure 7).

Figure 36 shows the two-photon excitation spectra of typical ¹L_b, (the origin), and ¹L_a, (at 1455.9 cm⁻¹), transitions. The thin line corresponds to the spectrum obtained using circularly polarized light, and the thick line corresponds to the spectrum obtained when perpendicular polarization was used. The respective Ω are 1.4 and 0.5, in good

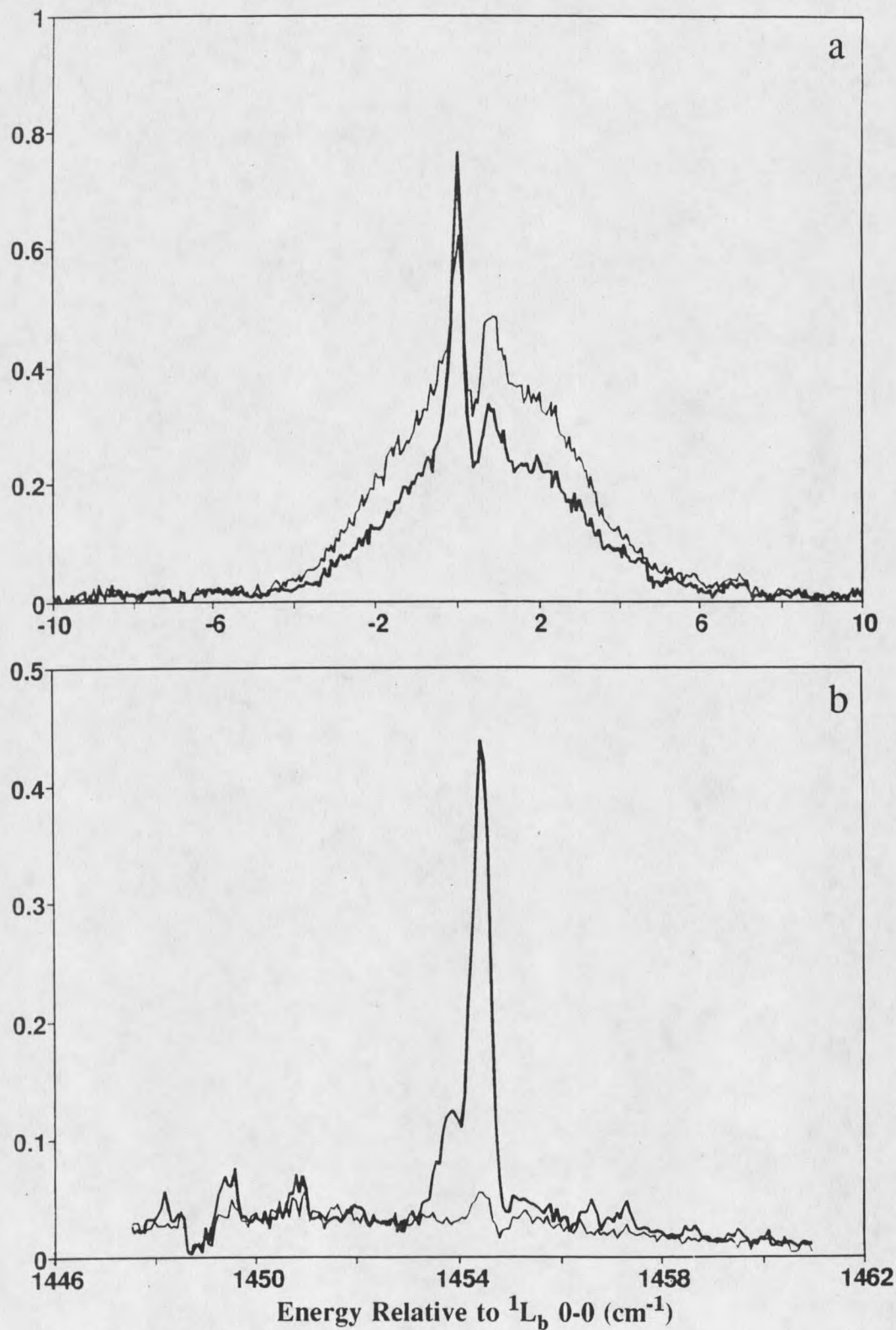


Figure 36. Two-photon excitation spectra of the 1L_b origin, (a), and an 1L_a transition, (b), of indole in supersonic jet.

agreement with the expected values reported in the BACKGROUND section. To obtain Ω , the two spectra, (using circularly and linear polarized light), obtained for each peak are integrated. The ratio of the areas gives Ω . The estimated error for the polarization ratios which will be reported in this section is ± 0.05 for the 1L_b origin of bare indole, ± 0.10 for the other peaks in bare indole and the 1L_b origin of the complex with methanol, and ± 0.2 for the 1L_a origin of indole+methanol. Also, the shape of the spectra are different for the different polarizations: the 1L_b spectrum is more intense with circularly polarized light, except at the Q branch¹⁵⁰, where the intensity with linearly polarized light is larger. The 1L_a spectrum contains mainly a Q branch, thus, the intensity with circularly polarized light is small, resulting in a low value for Ω . The shapes of the two photon spectra taken by Sammeth *et al.*¹²⁷ are significantly different from the shapes in our spectra, because the expansion conditions were different, resulting in a estimated temperature of ~ 1 K, compared to ~ 5 K in our experiments. When we changed the expansion conditions to allow for a better cooling, the shapes looked similar to those reported by Sammeth, where the half width at half maximum was ~ 2 cm^{-1} , which is significantly less than the ~ 5 cm^{-1} measured at 5 K.

The spectrum in Figure 35 serves as a background to which we can compare spectra of indole forming van der Waals complexes with solvents. In these latter ones, both complexes and bare indole appear in the spectra. Thus, comparing them to the spectrum in Figure 35 helps in identifying the transitions corresponding to the complex.

Figure 37 and 38 show the one-photon excitation spectra of indole+H₂O and indole+methanol, (the 1L_b origin in Figure 37, and the four highest peaks in Figure 38

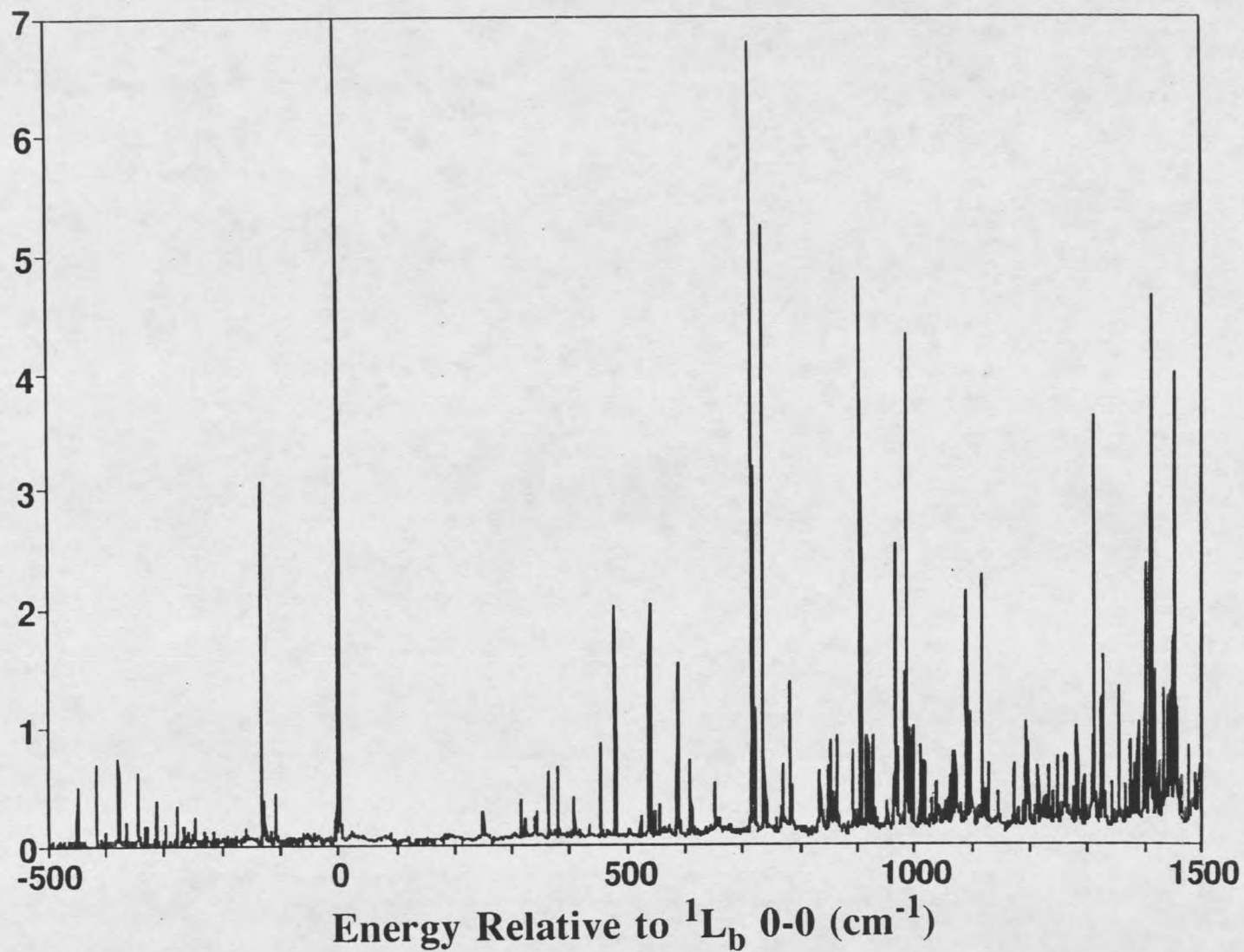


Figure 37. One-photon excitation spectrum of indole+H₂O in supersonic jet.

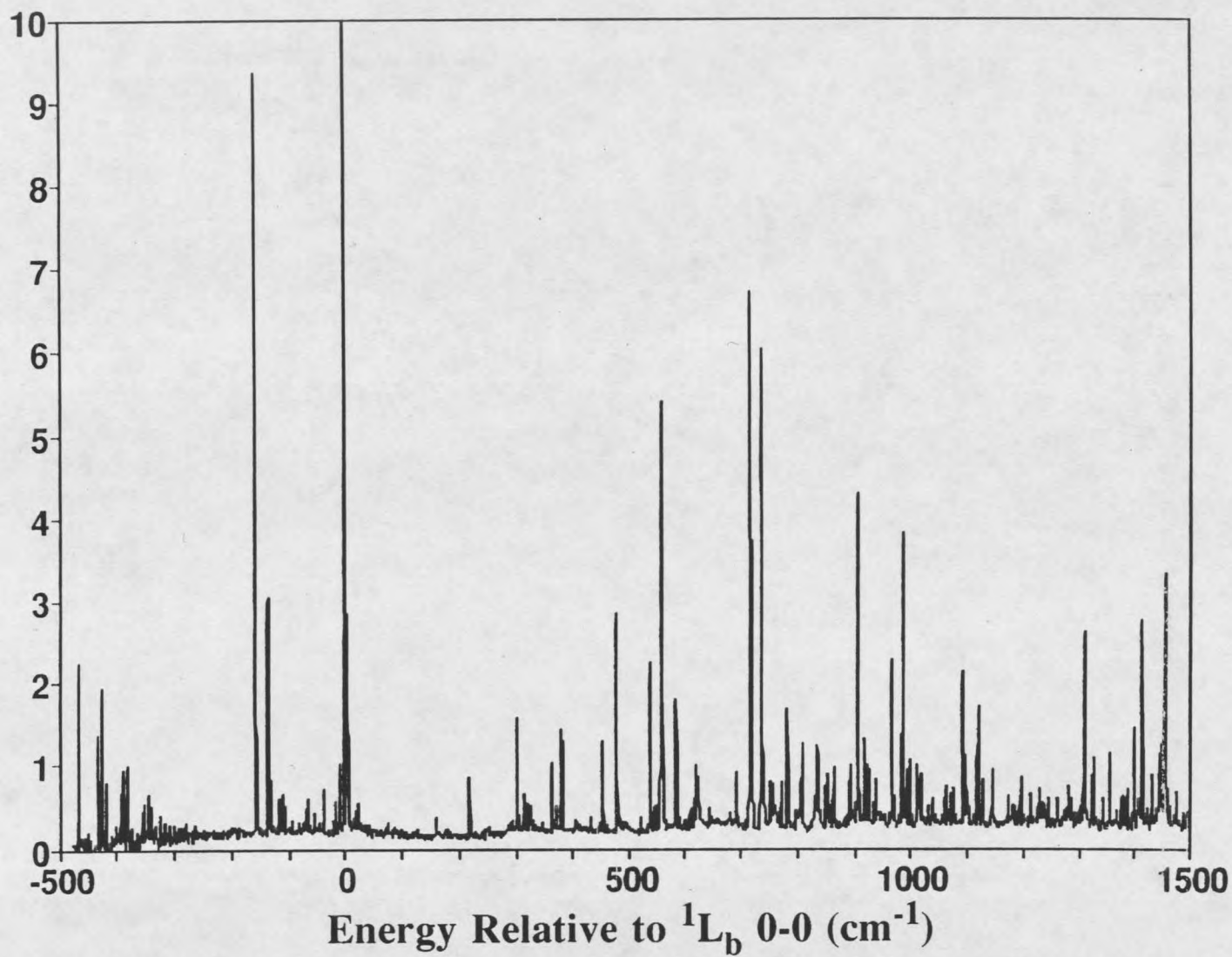


Figure 38. One-photon excitation spectrum of indole+methanol in supersonic jet.

are saturated). Visual comparison of these figures with Figure 35 reveals that there are significant differences, particularly in the region to the red of the 1L_b origin. (In Figure 35, the spectrum is not shown further to the red because no transition was observed in the region between 0 and -700 cm^{-1}). The reason for all these extra transitions can be traced to the formation of complexes between indole and the solvent with active intermolecular vibrations^{23,25,26,151-152}.

The Region of the 1L_b Origins of the Spectra of Complexes

Indole+Water

Figure 39 shows the region corresponding to the 1L_b origins of the complexes formed between indole and H_2O and indole and D_2O . The transitions at 0 cm^{-1} correspond to the 1L_b origin of bare indole and bare *d*-indole, respectively. It has been documented in the literature that there are two binding sites for these complexes^{25,26,55}, and this is evidenced in our spectra by the presence of two distinct origins in both traces in Figure 39. One of them, (which will be referred as complex II), appears at -449.4 cm^{-1} for indole+ H_2O and at -461.0 cm^{-1} for *d*-indole+ D_2O , while the other, (complex I), appears at -131.7 and -132.1 cm^{-1} , respectively. Wallace *et al.*²⁵ have used mass spectrometry to determine that both types of complexes correspond to the formula indole+(water)₁. Their behavior, however, is very different. Complex I shows a short Franck-Condon progression, (1 member), with a frequency of 24.4 cm^{-1} for the H_2O complex, and 23.2 cm^{-1} for the D_2O complex. Complex II shows a beautiful and long Franck-Condon progression with two easily identifiable vibrational modes with frequencies 35.1 and 48.5 cm^{-1} for indole+ H_2O and 33.6 and 47.8 cm^{-1} for

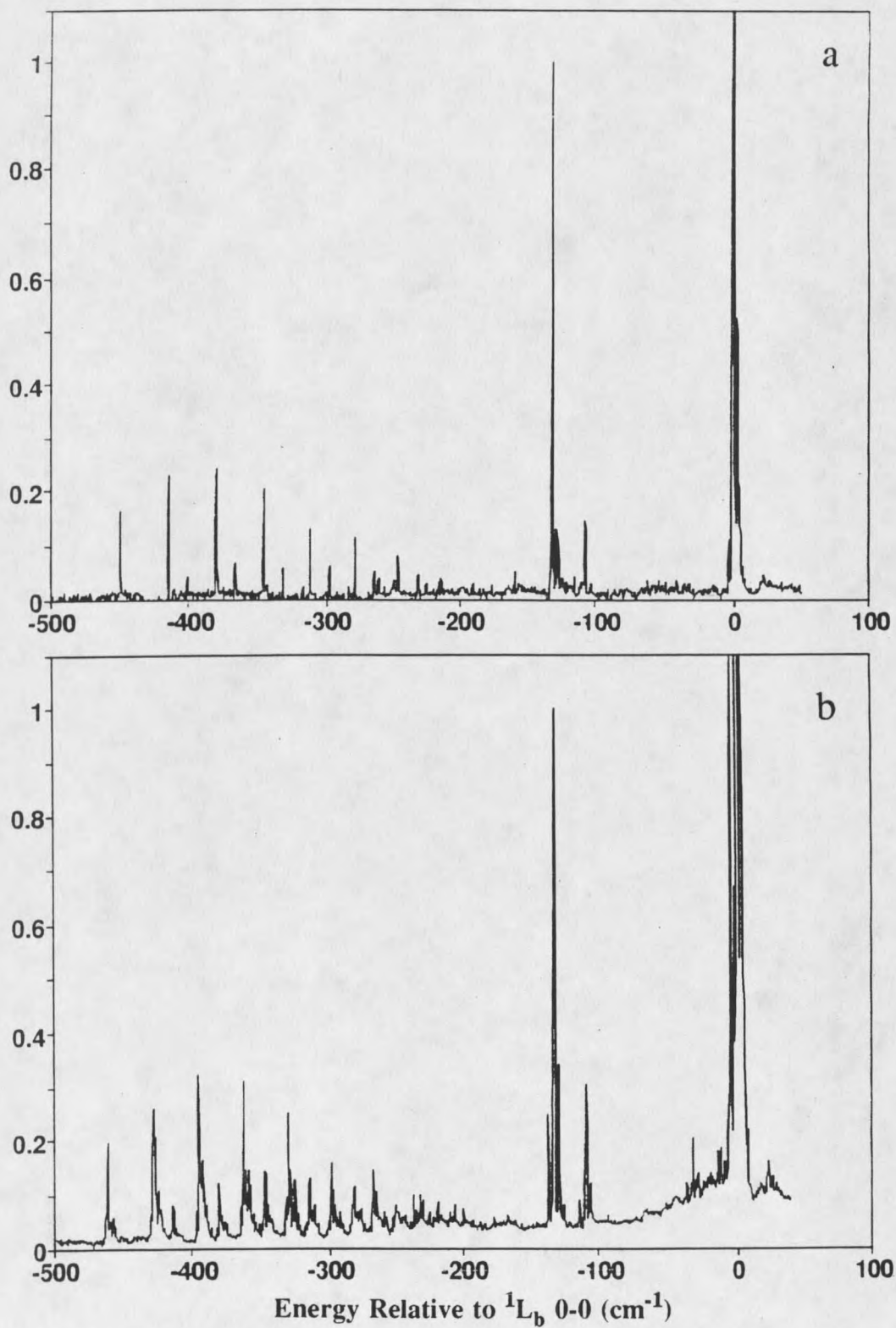


Figure 39. One-photon excitation spectra of the 1L_b origin region of indole+ H_2O , (a), and indole+ D_2O , (b), in supersonic jet.

d-indole+D₂O. Additionally, we believe we also have identified a third vibrational mode in the indole+H₂O complex with a frequency of 188.8 cm⁻¹. This mode has built upon a progression of mode I, with spacings of 35.2 and 34.7 cm⁻¹, very similar to the spacings of 35.1 and 34.6 cm⁻¹ built upon the origin.

The transitions in the indole+D₂O spectrum look much wider than those in the spectrum of indole+H₂O. For instance, inspection of the origin of the bare molecule, (although truncated), shows a different structure when comparing both graphs. The peak at 5 cm⁻¹ to the red of the origin in Figure 39b corresponds to the origin of *h*-indole. This is consistent with the values reported by Wallace *et al.*²⁵ which indicate that the ¹L_b origin shifts 5 cm⁻¹ to the blue upon deuteration in the 1 position, (see Figure 2). It seems clear, then, that although our starting materials were indole and D₂O, an exchange reaction took place in the nozzle and most of the indole was converted into 1-*d*-indole, (from now on referred as *d*-indole). Figure 40 shows a detailed spectrum of the origin of complex I in Figure 39b. It can be observed that it has more structure than its counterpart in Figure 39a, because of the different species present. Comparison of the relative intensities of the first two peaks, (starting from the left), to the relative intensities of the bare *h*-indole and *d*-indole peaks suggests that these two peaks correspond, respectively, to the ¹L_b origins of complex I of *h*-indole+D₂O and *d*-indole+D₂O. The third transition, (at -129.0 cm⁻¹ from the *d*-indole 0-0), is believed to correspond to the origin of complex I of *d*-indole+HDO. Rough estimates of the amount of *d*-indole formed suggest that it is certainly possible that enough HDO is present to form complexes.

The formation of trimers between an indole species, water, and helium, and other

dimers between the indole and water species adds further broadness to the peaks in the spectrum shown in Figure 39b. Most of the peaks are going to have this type of structure, with several complexes contributing to the total shape and intensity of the peak. In some cases, it is going to be difficult to separate the different transitions, but an attempt has been made to make assignments on the vibrational modes of the different complexes involved. Tables 7 through 10 show these assignments for *h*-indole+H₂O, *d*-indole+D₂O, *h*-indole+D₂O, and *d*-indole+HDO.

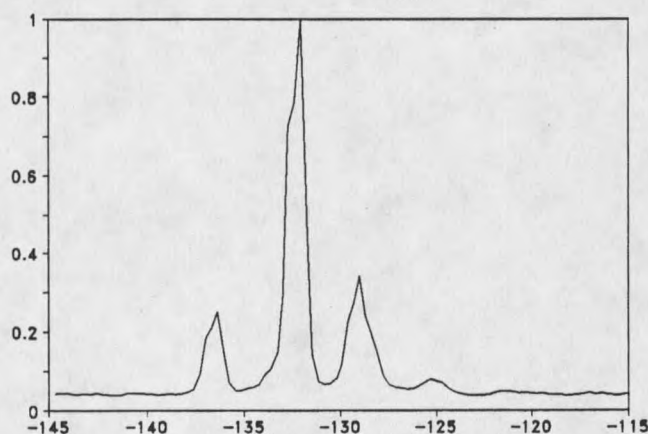


Figure 40. Origin of Complex I in the indole+D₂O system

Inspection of Figure 39 and Tables 7 to 10 reveals that similar features and spacings are present in spite of the significant difference in mass between H₂O and D₂O, which should result in a difference in the frequency of the vibrational intermolecular modes. The nature of the two complexes has been subject of debate in the literature. Wallace *et al.*²⁵ have suggested that both complexes could be hydrogen bonded to the hydrogen at the 1 position, but they would differ in the relative orientation of the acceptor with respect to the indole molecule. On the other hand, Tubergen and Levy²⁶ have

Table 7. *h*-indole + H₂O

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
-107.3	0.89	I, ν_1 (24.4 cm ⁻¹)
-131.7	6.09	I, 0-0
-190.7	0.18	II, $2\nu_1 + \nu_3$
-214.3	0.24	II, $7\nu_1$
-225.4	0.18	II, $\nu_1 + \nu_3$
-230.9	0.26	II, $5\nu_1 + \nu_2$
-246.2	0.48	II, $6\nu_1$
-249.2	0.21	II, $3\nu_1 + 2\nu_2$
-260.6	0.24	II, ν_3 (188.8 cm ⁻¹)
-264.2	0.33	II, $4\nu_1 + \nu_2$
-278.6	0.70	II, $5\nu_1$
-283.2	0.15	II, $2\nu_1 + 2\nu_2$
-297.5	0.38	II, $3\nu_1 + \nu_2$
-311.9	0.79	II, $4\nu_1$
-317.3	0.14	II, $\nu_1 + 2\nu_2$
-331.7	0.35	II, $2\nu_1 + \nu_2$
-345.3	1.24	II, $3\nu_1$
-352.4	0.06	II, $2\nu_2$
-366.0	0.40	II, $\nu_1 + \nu_2$
-379.7	1.48	II, $2\nu_1$
-400.9	0.26	II, ν_2 (48.5 cm ⁻¹)
-414.3	1.41	II, ν_1 (35.1 cm ⁻¹)
-449.4	1.00	II, 0-0

a Relative to the origin of complex II.

b The two complexes, (*h*-indole+(H₂O)₁), are labelled I and II.

Table 8. *d*-indole + D₂O

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
-28.6	0.70	-
-31.3	1.04	-
-36.0	0.61	-
-108.9	1.54	I, ν_1 (23.2 cm ⁻¹)
-132.1	8.5 ^c	I, 0-0
-206.8	0.40	II, $8\nu_1$
-236.8	0.49	II, $7\nu_1$
-250.6	0.40	II, $5\nu_1 + \nu_2$
-267.4	0.73	II, $6\nu_1$
-282.0	0.58	II, $4\nu_1 + \nu_2$
-298.5	0.80	II, $5\nu_1$
-314.4	0.66	II, $3\nu_1 + \nu_2$
-330.2	1.28	II, $4\nu_1$
-347.2	0.73	II, $2\nu_1 + \nu_2$
-362.2	1.56	II, $3\nu_1$
-366.7	0.14	II, $2\nu_2$
-379.8	0.62	II, $\nu_1 + \nu_2$
-394.7	1.64	II, $2\nu_1$
-413.2	0.41	II, ν_2 (47.8 cm ⁻¹)
-427.4	1.33	II, ν_1 (33.6 cm ⁻¹)
-461.0	1.00	II, 0-0

a Relative to the origin of complex II.

b The two complexes, (*d*-indole+(D₂O)₁), are labelled I and II.

c This line is saturated in the spectrum shown. The relative intensity with respect to the origin of complex II was obtained from another spectrum.

Table 9. *h*-indole + D₂O

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
-108.6	0.45	I, ν_1 (22.8 cm ⁻¹)
-131.4	1.26	I, 0-0
-295.7	0.26	II, $5\nu_1$
-327.4	0.35	II, $4\nu_1$
-359.3	0.36	II, $3\nu_1$
-391.5	0.36	II, $2\nu_1$
-424.2	0.33	II, ν_1 (33.6 cm ⁻¹)
-457.8	0.21	II, 0-0

a Relative to the origin of complex II of *d*-indole + D₂O.

b The two complexes, (*h*-indole+(D₂O)₁), are labelled I and II.

suggested the possibility of two different types of hydrogen bond between indole and water. Complex I would correspond to a species with a hydrogen bond between the solvent and the hydrogen at the 1 position, while complex II would have the solvent hydrogen-bonded to the π cloud of the indole ring. They have found that Complex I follows the pattern discovered by Hager and Wallace¹⁵³ where the hydrogen-bond accepting species induces a red shift which is linearly proportional to the proton affinity of the solvent. Complex II does not follow that pattern. We have performed calculations using molecular mechanics which find two minima, (supporting Levy's idea), for the indole+H₂O system in the places he suggested. (There are actually three minima, but two of them are equivalent since they correspond to bonding to the π cloud in either side of the ring). However, INDO/S calculations performed on those minima result in red shifts which do not agree with experimental values.

Table 10. *d*-indole + HDO

Spectral shift (cm ⁻¹)	Intensity ^a	Assignment ^b
-105.8	0.12	I, ν_1 (23.2 cm ⁻¹)
-129.0	1.72	I, 0-0
-225.5	0.33	II, $7\nu_1$
-259.1	0.33	II, $6\nu_1$
-277.5	0.35	II, $4\nu_1 + \nu_2$
-291.8	0.34	II, $5\nu_1$
-311.0	0.39	II, $3\nu_1 + \nu_2$
-324.8	0.64	II, $4\nu_1$
-345.8	0.42	II, $2\nu_1 + \nu_2$
-358.2	0.74	II, $3\nu_1$
-378.6	0.31	II, $\nu_1 + \nu_2$
-391.1	0.68	II, $2\nu_1$
-411.7	0.21	II, ν_2 (45.1 cm ⁻¹)
-423.7	0.55	II, ν_1 (33.1 cm ⁻¹)
-456.8	0.29	II, 0-0

a Relative to the origin of complex II of *d*-indole + D₂O.

b The two complexes, (*d*-indole+(HDO)₁), are labelled I and II.

Both models postulate that at least one complex, (I), must be hydrogen-bonded to H1. The sharp peak at 24.4 cm⁻¹ to the blue of the origin of this complex corresponds to an intermolecular vibrational mode, possibly involving a displacement of the solvent molecule towards and away from the N-H group. Indeed, the change in vibrational frequency from the D₂O to the H₂O complex, (although small in absolute value), agrees with the ratio shown in Equation 38,

$$\frac{\nu_1}{\nu_2} = \sqrt{\frac{m_2}{m_1}} \quad (38)$$

indicating that the solvent molecule as a whole is displaced instead of just the hydrogens. If only these atoms, (and not the oxygen), were moving, we would expect a larger change in the frequency: ν_{D_2O} should be $(m(D_2)/m(H_2))^{1/2}=1.41$ times smaller than ν_{H_2O} .

A similar analysis can be done on the vibrational modes of complex II. The frequencies of the first mode, (35.1 cm^{-1} for H_2O and 33.6 cm^{-1} for D_2O), show a similar ratio as those of complex I. The frequencies of the second mode are similar in both complexes, (48.5 and 47.8 cm^{-1} respectively), but they are within experimental error of what one would expect if the whole molecule were moving. (If only the hydrogens were moving, there should be a 40% difference in frequencies, (see above), which is clearly not the case).

According to the Born-Oppenheimer approximation, the electrons move much faster than the nuclei. Thus, when the molecule absorbs a photon and gets promoted to the excited state, the nuclei remain virtually motionless in this interval, ($\sim 10^{-14}$ sec, according to Steinfeld¹¹⁰). Therefore, the molecule reaches the excited state but still retains the ground state geometry, even when the equilibrium geometries for both states are different. This is the basis of the so called Franck-Condon principle^{154,155}. According to this principle, the intensity of a transition between any two vibrational states is equal to the square of the vibrational overlap integral, $|\langle v' | v'' \rangle|^2$, which is also referred as a Franck-Condon Factor, $(f_{v'' \rightarrow v'})$.

From the relative intensities, (and spacing), of the members of a progression starting in the vibrational ground state of the electronic S_0 , we can recover information about the relative displacement between the equilibrium geometries of both electronic states¹⁵⁶.

$$I = \frac{\lambda^n}{n!} e^{-\lambda} = f_{0-n} \quad (39)$$

where λ is a parameter that can be obtained from the ratio of the intensities of the origin and first member of the progression:

$$\frac{f_{0-1}}{f_{0-0}} = \frac{\lambda e^{-\lambda}}{e^{-\lambda}} = \lambda \quad (40)$$

From Table 7, we can see that this ratio is 1.41 for the first vibrational mode of complex II of *h*-indole+H₂O. The dimensionless displacement, s , (which is a measure of the distance between the equilibrium geometries of both electronic states), is equal to $(2\lambda)^{1/2}$. Therefore, in this case, s is $(2 \times 1.41)^{1/2} = 1.68$. From s , we can calculate the net displacement, ΔQ , using Equation 41,

$$\Delta Q = s \sqrt{\frac{\hbar}{\omega \cdot \mu}} = 1.68 \sqrt{\frac{33.715 \text{ \AA}^2 \text{ amu cm}^{-1}}{35.1 \text{ cm}^{-1} 15.6 \text{ amu}}} = 0.42 \pm 0.04 \text{ \AA} \quad (41)$$

where μ , 15.6 amu, is the reduced mass of the indole+H₂O system. The error in the displacement has been obtained by introducing in Equation 41 the error in the

measurement of frequencies and relative intensities.

This analysis can also be performed for the other mode of complex II and the single mode of complex I, yielding displacements of $0.15 \pm 0.02 \text{ \AA}$ and $0.16 \pm 0.02 \text{ \AA}$, respectively.

In contrast, molecular mechanics predicts a displacement of the center of mass of $0.08 \text{ \AA}$ for complex I, if the molecule is excited to the 1L_b state, and $0.13 \text{ \AA}$ if it is excited to the 1L_a state. It predicts displacements of 0.18 and $0.26 \text{ \AA}$, respectively, for complex II.

As mentioned previously, INDO/S calculations on these geometries yielded mixed results. The predicted red shift for complex I is 134 cm^{-1} for the 1L_b transition and 719 cm^{-1} for 1L_a ; while the predicted red shifts for complex II are 23 and 216 cm^{-1} , respectively.

Another interesting question is that of the anharmonicity of the potential energy surfaces for the complexes. In this respect, complex II for the indole+H₂O system, has a mode, ν_1 , with up to six overtones in the spectrum. The presence of so many transitions makes it a likely candidate for this type of study.

If the potential energy surface were harmonic, the energy, G_v , of any vibrational state v from the bottom of the potential energy well would be

$$G_v = \left(v + \frac{1}{2} \right) \omega'_e \quad (42)$$

where the prime indicates that we are referring to the excited state, and ω_e represents the

spacing between levels.

However, if the potential is not harmonic, other terms have to be added to this expression¹⁵⁷:

$$G_v = \left(v + \frac{1}{2}\right) \omega_e' - \left(v + \frac{1}{2}\right)^2 \chi_e' \omega_e' + \left(v + \frac{1}{2}\right)^3 y_e' \omega_e' + \dots \quad (43)$$

where χ_e' and y_e' are anharmonicity terms. Calculation of ω_e' , $\chi_e' \omega_e'$ and $y_e' \omega_e'$ is fairly straightforward if enough overtones are present in the spectrum.

Table 11 shows the first, second, and third order differences for the transitions corresponding to the first intermolecular vibrational mode of complex II for indole+H₂O. The average value of the third order differences, Δ_3 , gives the value for $y_e' \omega_e'$. Using this calculated value, we can manipulate the second order differences, Δ_2 , in order to obtain $\chi_e' \omega_e'$. Finally, plugging these two values into our list of the first order differences, Δ_1 , we can get ω_e' .

With the differences shown in Table 11, we have calculated the following values: $\omega_e' = 35.72 \pm 0.16 \text{ cm}^{-1}$, $\chi_e' \omega_e' = 0.27 \pm 0.02$, and $y_e' \omega_e' = 0.000 \pm 0.003$. This means, that the third order correction for anharmonicity, y_e' , is 0.0000 ± 0.0001 , while the second order correction, χ_e' , is 0.0076 ± 0.0006 . Thus, we can see that the potential energy is close to harmonic for the first 7 vibrational levels.

The spacing between two consecutive levels, v_x and v_x+1 , is:

$$\Delta G = 35.72 - 0.54(v_1' + 1) \text{ cm}^{-1} \quad (44)$$

Table 11. Spacings between the members of the progression of the first intermolecular vibrational mode of complex II for the indole+H₂O system.

Spec. shift (cm ⁻¹)	Δ_1^a	Δ_2^a	Δ_3^a
-449.4			
-414.3	35.1		
-379.7	34.6	-0.5	
-345.3	34.4	-0.2	0.3
-311.9	33.4	-1.0	-0.8
-278.6	33.3	-0.1	0.9
-246.2	32.4	-0.9	-0.8
-214.3	31.9	-0.5	0.4

a Δ_1 refers to the spacing between two consecutive transitions, Δ_2 refers to the differences between two consecutive Δ_1 s, and Δ_3 refers to the difference between two consecutive Δ_2 s.

Indole+Methanol

Comparison of Figures 37 and 38 shows that the spectrum of indole+H₂O and indole+methanol share the fact that two sets of transitions, corresponding to two different complexes, are present. However, possibly because of the large size of the methanol molecule as compared to H₂O, which results in a larger number of degrees of freedom, the details of the spectra are different.

Figure 41 shows the region of the ¹L_b origin of the spectrum of indole+methanol. Wallace *et al.*²⁵ have identified two complexes with formula indole+(CH₃OH)₁, labelled I and II, which correspond to equivalent geometries to those seen for indole+water, and one complex with formula indole+(CH₃OH)₂. Their identification is based on two-color photoionization followed by detection of the ions in a quadrupole mass spectrometer.

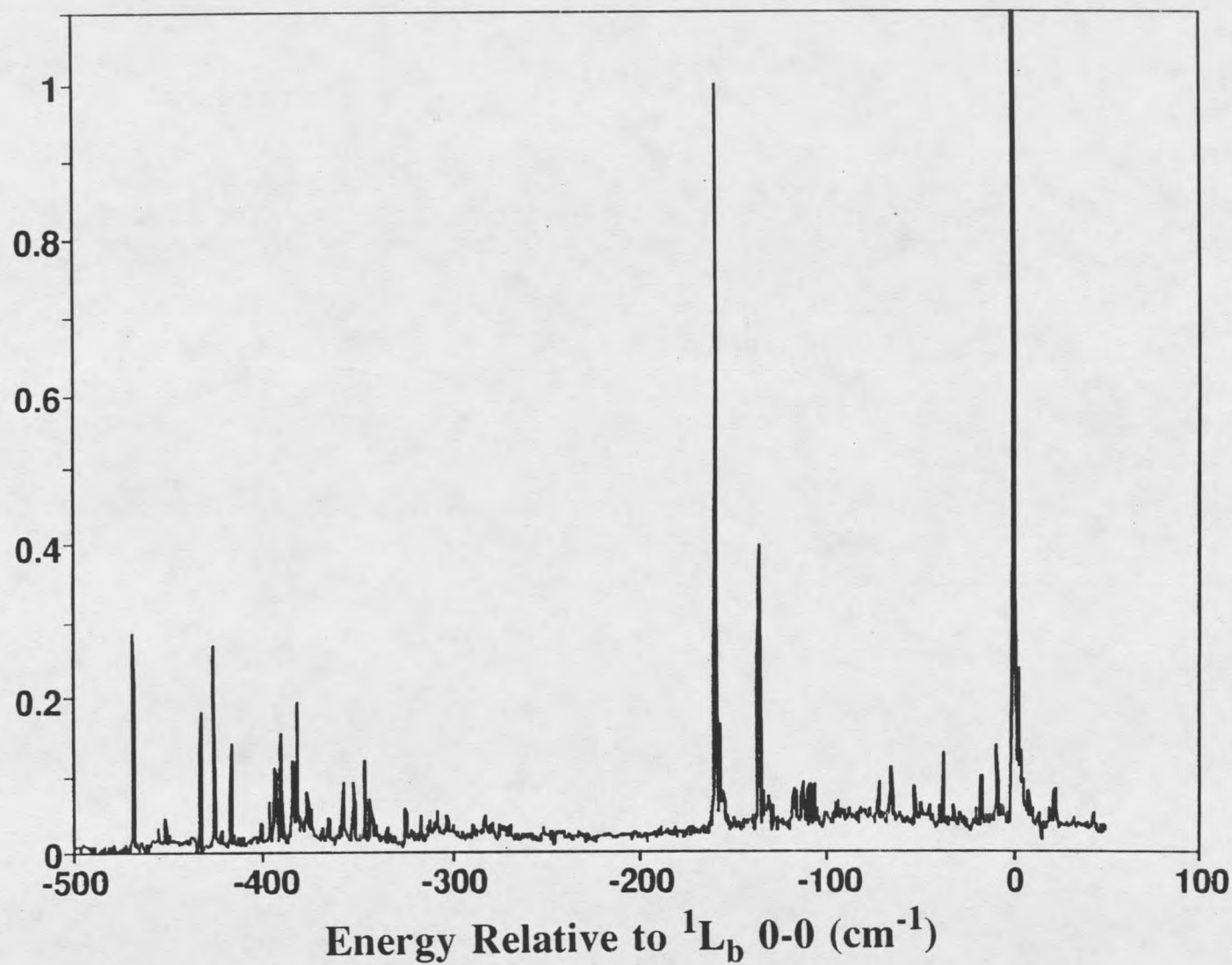


Figure 41. One-photon excitation spectrum of the 1L_b origin region of indole+methanol in supersonic jet.

Table 12 shows a list of the observed transitions and intensities relative to the origin of complex I. The assignments of the transitions are also included. Apparently, the amount of complex II formed is very dependent on the expansion conditions in the jet, (see Appendix B), because we have a ratio of intensities for the origins of complex II and I of 0.29, while from the spectrum shown in reference 25, this ratio can be estimated to be between 0.05 and 0.10. We have done experiments where the amount of methanol in the lines is optimized to produce the largest possible amount of complex I. When this is done, the ratio goes down to ~0.05, (independently of the nozzle distance), which is compatible with the numbers obtained by Wallace *et al.*

Several explanations can be presented to explain this difference. On one hand, we can think that this phenomenon is related to the binding energy of the complex. It is expected that this energy is larger for complex I. Then, it is reasonable to expect that certain expansion conditions may be affecting complex II by knocking off solvent molecules from the complexes. It can also be argued that changing the expansion conditions effectively changes the amount of methanol in the lines. Thus, if complex II were not a 1:1 complex but a 1:2 complex, optimizing the formation of complex I, (a 1:1 complex), would certainly result in a decrease of the relative population of complex II. Although Wallace and coworkers have detected the ions formed after excitation to the modes of complex II in the 1:1 mass channel, it cannot be completely ruled out that, because of the low binding energy, the complex did not lose a solvent molecule before detection. This behavior has been observed for benzene+(H₂O)₂¹³⁷, where most of the complex is observed in the 1:1 mass channel.

Table 12. 1L_b origin region for the indole+methanol complex.

Freq. shift (cm ⁻¹)	Intensity ^a	Assignment ^b	Freq. shift (cm ⁻¹)	Intensity ^a	Assignment ^b
-9.5	0.14		-343.1	0.07	II, $\nu_3+\nu_4$
-17.2	0.10	X, ν_2 (48.6)	-346.4	0.12	II, $\nu_1+\nu_5$
-38.3	0.13	X, $2\nu_1$	-352.5	0.09	II, ν_7 (116.4)
-52.9	0.09	X, ν_1 (12.9)	-357.4	0.09	II, $\nu_1+\nu_4$
-65.8	0.11	X, 0-0	-365.6	0.04	II, $2\nu_3$
-72.0	0.09		-374.7	0.06	II, $\nu_2+\nu_3$
-107.2	0.09		-376.2	0.08	II, ν_6 (92.7)
-109.4	0.09		-382.0	0.19	II, ν_5 (86.9)
-112.8	0.09	I, $2\nu_1$	-384.1	0.12	II, $2\nu_2$
-116.5	0.08		-390.2	0.16	II, $\nu_1+\nu_2$
-117.7	0.08		-392.9	0.10	
-131.3	0.07	c	-394.1	0.11	II, ν_4 (75.4)
-136.2	0.40	I, ν_1 (23.7)	-396.6	0.07	II, $2\nu_1$
-159.9	1.00	I, 0-0	-401.1	0.04	
-302.9	0.05	II, $\nu_3+\nu_7$	-417.2	0.14	II, ν_3 (51.7)
-308.4	0.06	II, $\nu_2+\nu_7$	-426.3	0.27	II, ν_2 (42.6)
-316.9	0.05	II, $\nu_1+\nu_7$	-432.6	0.18	II, ν_1 (36.3)
-324.8	0.06	II, $\nu_3+\nu_6$	-451.4	0.04	c
-342.5	0.05	II, $3\nu_2$	-468.9	0.29	II, 0-0

a. Intensity relative to the origin of Complex I.

b. Assignments based on Ref. 25. There are two complexes of the form Indole-(CH₃OH)₁, labelled I and II, and one of the form Indole-(CH₃OH)₂, labelled X. The numbers in parentheses correspond to the frequency in cm⁻¹ of the vibrational mode.

c. These peaks possibly correspond to the complex Indole-(H₂O)₁, formed because of water impurities remaining from previous experiments.

In this work just referenced, Wallace *et al.* assigned three vibrational modes to complex II, one to complex I, and two to the detected 1:2 complex, (labelled X in Table 12). In our experiments, we have detected at least 7 modes for complex II, and one and at least two for complexes I and X, respectively, (see Table 12). As a result of the increased intensity of the transitions in our spectra, we have positively identified vibrational modes in complex II with the following frequencies: 36.3, 42.6, 51.7, 75.4, (this is the average of a split peak), 86.9, 92.7, and 116.4 cm^{-1} . There is another transition, at 67.8 cm^{-1} from the origin of the complex, which cannot be assigned as an overtone or combination band of the other modes. However, its low intensity, which would make impossible the detection of combination bands with other modes, does not allow for a positive identification.

Complex I has only one identifiable vibrational mode, with frequency 23.7 cm^{-1} , which even presents an overtone. There are also four other transitions in its vicinity that cannot be assigned.

Finally, we have detected the same four transitions that Wallace *et al.*²⁵ assigned to the 1:2 complex. In their vicinity, there are two other transitions: one is 6.2 cm^{-1} to the red of their proposed origin, and the other is 56.3 cm^{-1} to the blue. However, since we do not have the capability to assign masses to these complexes, an attempt to associate these two transitions with this complex would be guesswork on our part.

Comparison of Figures 39a and 41 shows another interesting phenomenon. The origins of complex I have a fairly large satellite peak corresponding to the trimer indole-(solvent)₁-He. However, the trimer peaks are conspicuously absent, or are small,

in the peaks corresponding to complex II. This supports the idea of solvent bonding to the π cloud in complex II, because that geometry would hinder the access of helium to the binding site over the ring, thus resulting in a smaller, (or null), intensity for the associated trimer.

In this discussion, we have been assuming, (as it was assumed in the literature^{25,26,55,152}), that these transitions to the red of the 1L_b origin correspond to the 1L_b state of the complexes. This assumption can be tested using two photon spectroscopy. Figure 42 shows the two-photon excitation spectrum of the origin of complex I for indole+methanol, (once again, the thin and thick lines correspond to the fluorescence spectra obtained using circularly and linearly polarized light). Integration of each line results in a polarization ratio of 1.4, consistent with the 1L_b character. Visual comparison of this figure with Figure 36a reveals that the shape of the spectrum is similar to that of the parent transition, (although the signal to noise ratio is worse as a result of a lower intensity for this transition).

Attempts to measure the two-photon spectrum of the origin of complex II were unsuccessful. The expansion conditions necessary to have a large enough two-photon cross section, (see METHODS section), did not result in a significant amount of complex II, as evidenced by one-photon studies. Perhaps, at 0.5 cm from the nozzle, (the distance used in two-photon experiments), the molecules have enough kinetic energy to break the weakly bound complex II. Evidence of the 1L_b nature of this complex will be provided in the next section.

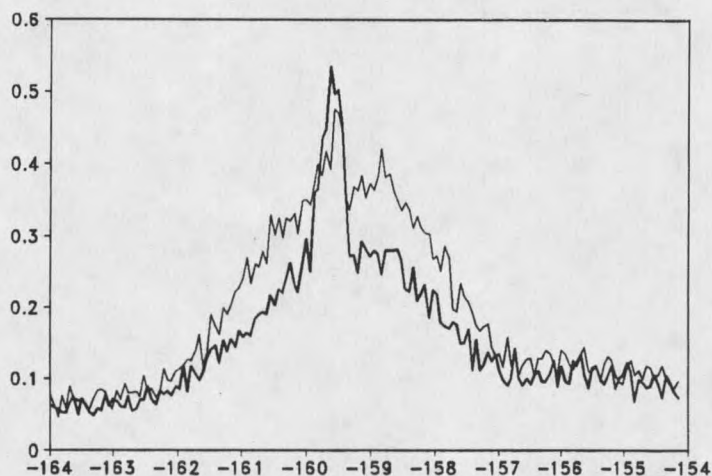


Figure 42. Two-photon spectrum of the origin of complex I of indole+(CH₃OH)₁

The Region of the "¹L_a Origins" of the Spectra of Complexes

The study of the region surrounding the ¹L_a origins of indole, (at 455 and 480 cm⁻¹ to the blue of the ¹L_b origin), is the object of this section. This study is more complex than that of the ¹L_b origin region since there are a lot of bare molecule transitions intermixed with those corresponding to the complexes. On the other hand, the intensity of the bare molecule peaks in this area is much smaller than that of the origin. This means that, in most cases, only one line, that corresponding to an indole vibrational mode of complex I, will be seen. Therefore, (except for a few cases in indole+methanol), complex II lines will not be seen. Lines corresponding to intermolecular vibrational modes of complex I will not be present in the spectra, either.

Tables 13, 14, and 15 show the transitions for indole+H₂O, *d*-indole+D₂O, and indole+methanol, and their assignments. It must be pointed out that the transitions in

Table 14 are referred to the origin of *d*-indole, which is 5 cm^{-1} to the blue of the origin of *h*-indole²⁷. Most of the transitions in *d*-indole show a similar spectral shift to their counterparts in *h*-indole, (with a difference of $2\text{-}4\text{ cm}^{-1}$). However, Wallace *et al.*²⁷ reported, (in agreement with our own experiments), that the peak assigned by Callis *et al.*³⁵ as the first $^1\text{L}_a$ origin of indole appears in *d*-indole at 442 cm^{-1} to the blue of the $^1\text{L}_b$ origin. This means a shift of 13 cm^{-1} with respect to its *h*-indole counterpart.

Table 13. Indole+H₂O. Assignments for the $^1\text{L}_a$ origin region.

Spec. shift (cm^{-1})	Intensity ^a	Assignment ^b	Shift ^c (cm^{-1})
185.0	0.05	42_0^2	-131.6
250.7	0.15	29_0^1	-129.8
326.4	0.17	$^1\text{L}_a$ ("455")	-128.5
340.1	0.23	$^1\text{L}_a$ ("480")	-139.2
342.3	0.23		
408.8	0.29	27_0^1	-131.0
587.8	1.00	26_0^1	-130.0
611.6	0.44	37_0^2	-125.0
653.8	0.36	"784"	-130.0
837.2	0.50	"968"	-131.0

a Relative to the 26_0^1 transition of the complex.

b Indicates the assignment in the spectrum of bare indole to whom the transition is related to.

c Relative shift to the *parent* transition in the spectrum of bare indole.

From Table 13, we can see that all of the $^1\text{L}_b$ transitions shift $\sim 130\text{ cm}^{-1}$ with respect to their parent transition, with the exception of the 37_0^2 transition, which only shifts 125 cm^{-1} . In contrast, the transition associated with the $^1\text{L}_a$, (at 480 cm^{-1}), origin

shifts 139.2 cm^{-1} , and it is split. This is consistent with the expectation that the 1L_a levels are shifted more by a polar solvent than 1L_b levels are. However, this difference, $\sim 9\text{ cm}^{-1}$, is minute if we compare it to the shifts calculated by INDO/S, (see Indole+Water section). In these calculations, the predicted red shifts were 134 and 719 cm^{-1} for 1L_b and 1L_a respectively. We will discuss this behavior later in this section.

Table 14. Indole + D_2O . Assignments for the 1L_a origin region.

Spec. shift (cm^{-1})	Intensity ^a	Assignment ^b	Shift ^c (cm^{-1})
241.2	0.28	29_0^1	-129.8
288.5	0.13		
332.1	0.37	1L_a ("480")	-144.2
401.2	0.16	27_0^{1d}	-132.9
406.6	0.32	27_0^1	-133.5
578.6	1.00	26_0^1	-133.5
606.5	0.45	37_0^2	-126.3
687.6	0.39		
693.4	0.47		
799.9	0.34	"929"	-130.4
822.3	0.38		

- a Relative to the 26_0^1 transition of the complex.
- b Indicates the assignment in the spectrum of bare indole to whom the transition is related to.
- c Relative shift to the *parent* transition in the spectrum of bare indole.
- d This peak corresponds to the complex *h*-indole + D_2O .

The other 1L_a origin, (at 455 cm^{-1} in bare indole), shifts less than the one just mentioned, $\sim 128.5\text{ cm}^{-1}$. The different spacing in solution could be, in principle,

Table 15. Indole + methanol. Assignments of the 1L_a origin region.

Spec. shift (cm ⁻¹)	Intensity ^a	Assignment ^b	Shift ^c (cm ⁻¹)
160.0	0.07	42_0^2 (I)	-154.8
219.9	0.16	29_0^1 (I)	-158.5
243.8	0.05	$29_0^1 + \nu_1$ (I)	-158.5+23.9
250.7	0.05	26_0^1 (II)	-465.2
293.0	0.07	$26_0^1 + \nu_2$ (II)	-465.2+42.3
302.4	0.29	1L_a ("480") (I)	-175.8
320.4	0.10	1L_a ("455") (I)	-132.6
325.8	0.10	1L_a ("480")+ ν_1 (I)	-175.8+23.4
370.4	0.09		
381.2	0.24	27_0^1 (I)	-156.7
404.4	0.07		
490.0	0.06	"968" (II) (?)	-476.4
557.4	1.00	26_0^1 (I)	-158.5
581.1	0.21	$26_0^1 + \nu_1$ (I)	-158.5+23.7
583.7	0.33	37_0^2 (I)	-153.9
614.5	0.11	$36_0^1 39_0^1$	-156.8
619.0	0.19	"784"	-164.8
679.2	0.08		
692.4	0.17		
751.2	0.15	"907.5" (I)	-154.4
806.6	0.23	"968.2" (I)	-159.8
835.5	0.23	36_0^2	-156.8
1277.6	0.09	"1435.4" (?)	-157.8

a Relative to the 26_0^1 transition of the complex.

b Indicates the assignment in the spectrum of bare indole to whom the transition is related to. The roman numbers in parentheses indicate the nature of the complex.

c Relative shift to the *parent* transition in the spectrum of bare indole.

attributable to the coupling of an *unique* 1L_a state with a dark 1L_b state, as previously stated. If we assumed that complexation, (or *d*- substitution, as in the case of *d*-indole), shifted the 1L_b dark state and the 1L_a origin differently, the resulting coupling of states would not be the same as it was for bare indole. Therefore, the spacing between the observed transitions would be different. However, a linear variation analysis of the coupling of states reveals that a two level system cannot explain the behavior of these two transitions in the indole+H₂O complex if 1L_a states shift more than 1L_b states, (it cannot explain it for indole+methanol, either). If we assume that complexation shifts the dark 1L_b state slightly less than the 1L_b origin, as is seen for out-of-plane modes, (see below), the new positions of the lines can be explained by linear variation on a two level system, but the calculated intensities are very different from those seen in the spectra. (It is predicted that both transitions should have roughly the same intensity, both in water and in methanol).

In Table 15, it would be very interesting to observe a similar pattern for the red shift of the 1L_a origins, but this is not the case. The peak corresponding to the second 1L_a origin shifts even more, ($\sim 144.2 \text{ cm}^{-1}$, or $\sim 11 \text{ cm}^{-1}$ more than the 1L_b transitions), but the peak corresponding to the other 1L_a origin is absent, (or is lost in the complicated spectrum arising from the coexisting of isotopic species). In fact, the system is not necessarily similar to indole+H₂O because isotopic substitution, (yielding *d*-indole), is affecting the ring modes.

Another important feature present in Table 13 is that the peak associated with the possible 1L_a line at 784 cm^{-1} only shifts 130 cm^{-1} . Sammeth¹²⁷ reports a polarization

ratio of 0.9 for this transition, in agreement with our value of 1.0. This polarization value is intermediate between what we would expect from pure 1L_a and pure 1L_b transitions. Therefore, based on the red shift upon complexation, assignment of this peak as a 1L_a transition, (see Table 6), seems to be wrong. Unfortunately, we could not obtain a two-photon of the corresponding transition in the indole+methanol complex.

The spectrum of the complex between indole and methanol is richer in transitions than that of indole+H₂O. Apparently, it is easier for indole to form a large number of 1:1 complexes with methanol. For the complex with water, if one increases the amount of H₂O in the lines, (see METHODS section), the result is not an increased number of 1:1 complexes, but an increase in the number of large solvent clusters which cause the spectrum to look like the spectrum of indole in solution. The large number of complexes formed with methanol results in a larger intensity for the transitions, allowing for peaks corresponding to complex II or to intermolecular modes of both complexes to be observed.

Most of the 1L_b peaks shift $\sim 158\text{ cm}^{-1}$, while one of the 1L_a origins shift 175.8 cm^{-1} and the other shifts only 132.6 cm^{-1} , resulting in a behavior similar to, (but more exaggerated), than that of the indole+H₂O complex.

Figure 43 shows a region of the one-photon excitation spectrum of indole+methanol. The transitions marked with a dot correspond to vibrational modes of complex II. These transitions are small, but they appear consistently at the same frequency in all the spectra taken. The first of them corresponds to the 26_0^1 mode of complex II, while the second is an intermolecular vibration, (mode ν_2), built upon the

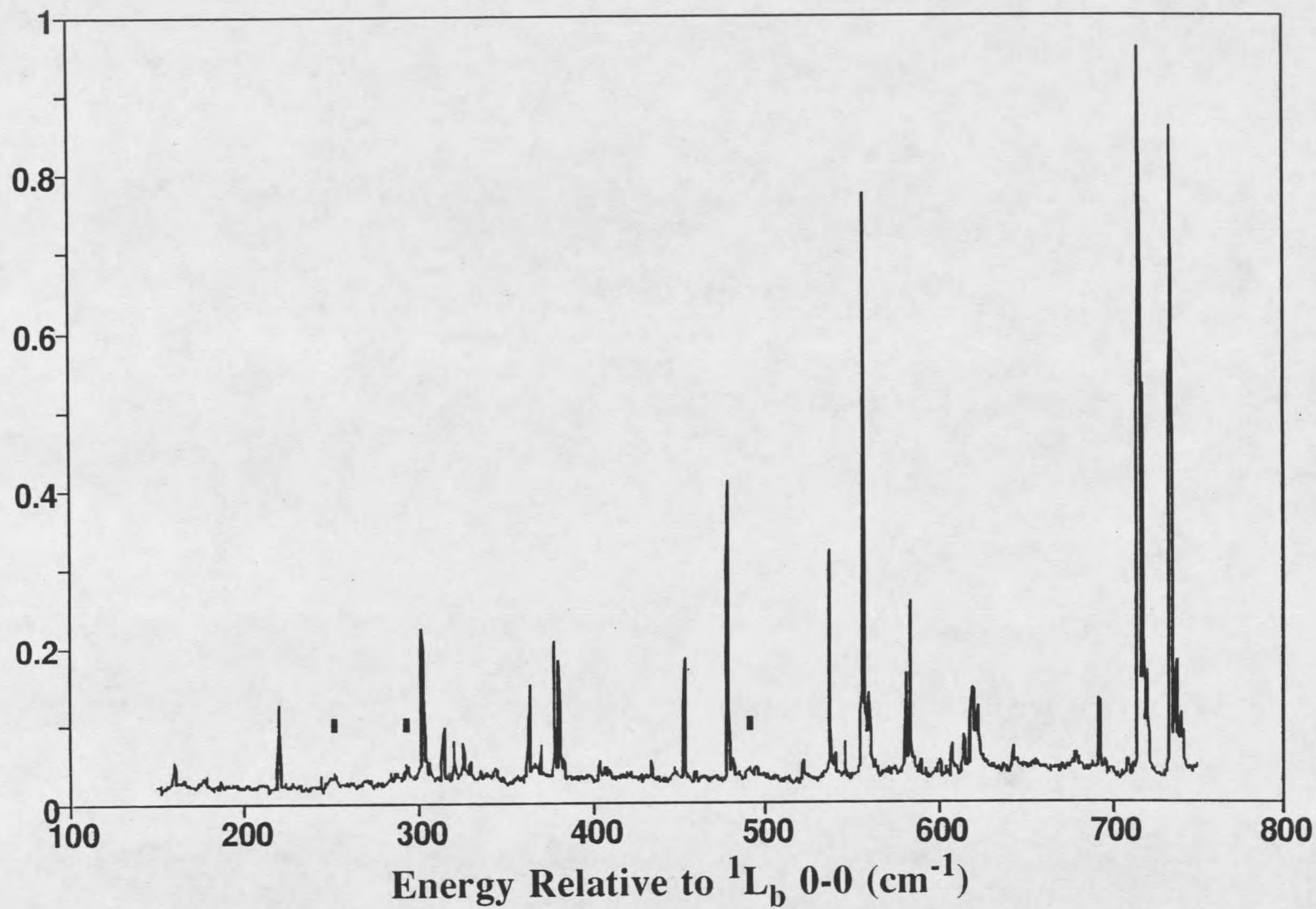


Figure 43. One-photon excitation spectrum of the " 1L_a origin" region of indole+methanol in supersonic jet.

previous transition. The third one possibly corresponds to a vibration of complex II equivalent to the mode labelled as "968" in the spectrum of bare indole, although the shift is slightly larger than for the 1L_b origin: -476 cm^{-1} vs -469 cm^{-1} . In the previous section, it was mentioned that the origin of complex II could not be assigned to the 1L_b by means of two-photon spectroscopy because of the low intensity of the transition. Thus, one could conceivably believe this to be the 1L_a origin of the complex, since calculations predict a 719 cm^{-1} red shift, (in water), and this transition, (also in water), is at $\sim 930\text{ cm}^{-1}$ to the red of the 1L_a origin of bare indole. However, the fact that these ring-vibrational modes are seen indicates with almost complete certainty that they correspond to a second complex and the origin at -460 cm^{-1} to the red of the bare indole origin must be 1L_b in nature. For it to be 1L_a , one would have to postulate that at least two vibrational modes, (or more, since "968" is assigned by Wallace *et al.*²⁷ as a mixture of out-of-plane modes), of the 1L_a state are identical, (within 5 cm^{-1}), to their counterparts in the 1L_b state, which is highly unlikely.

In both indole+water and indole+methanol spectra, there are some transitions which shifted $\sim 5\text{ cm}^{-1}$ less with respect to their parent transitions than the rest. Careful inspection reveals that all of them correspond to out-of-plane vibrational modes. Furthermore, with the exception of the 42_0^2 level of indole+H₂O, and the level in the indole+methanol spectrum equivalent to the one at 968.2 cm^{-1} in the spectrum of bare indole, (which Wallace *et al.*²⁷ assigned as corresponding to a mixture of out-of-plane modes), all transitions corresponding to out-of-plane modes shift less than the in-plane ones.

It is interesting to note that the 41_0^2 level cannot be seen in any of the complexes studied. This does not seem to be an intensity problem, since there are other transitions which should be less intense and are seen, (like the 42_0^2 level). This is a puzzling result, and needs to be investigated in more detail.

Finally, it has to be pointed out that no transition for any of the complexes is observed above 850 cm^{-1} to the blue of the 1L_a origin of bare indole, with the possible exception of a transition observed at 1278 cm^{-1} in the spectrum of indole+methanol. This transition would represent a red shift of 157.8 cm^{-1} with respect to the bare molecule transition at 1435.4 cm^{-1} , which is certainly reasonable. The fact that no other transitions are seen can be explained by considering that the energy of excitation could be transferred to the bond between the two species of the complex resulting in dissociation. In the observed transition, the ring vibration is probably not coupled to the "bond breaking", and the energy of excitation is not available to knock the solvent off the complex. It is also possible that some other transitions are present, but cannot be seen because there are so many transitions corresponding to the bare molecule that they just overlap these latter ones.

Figure 44 shows a 250 cm^{-1} region, (in the neighborhood of the 1L_a origins), for indole and indole complexed with methanol, H_2O , and D_2O . In these last three cases, the spectra have been shifted by 132, 132 and 160 cm^{-1} to the blue, respectively, (that is, a distance equal to that between the origin of complex I and the bare indole origin in each of the solvents). Also, in these spectra, the transitions corresponding to the bare molecule have been deleted, in order to not interfere in the picture. Finally, in the spectrum

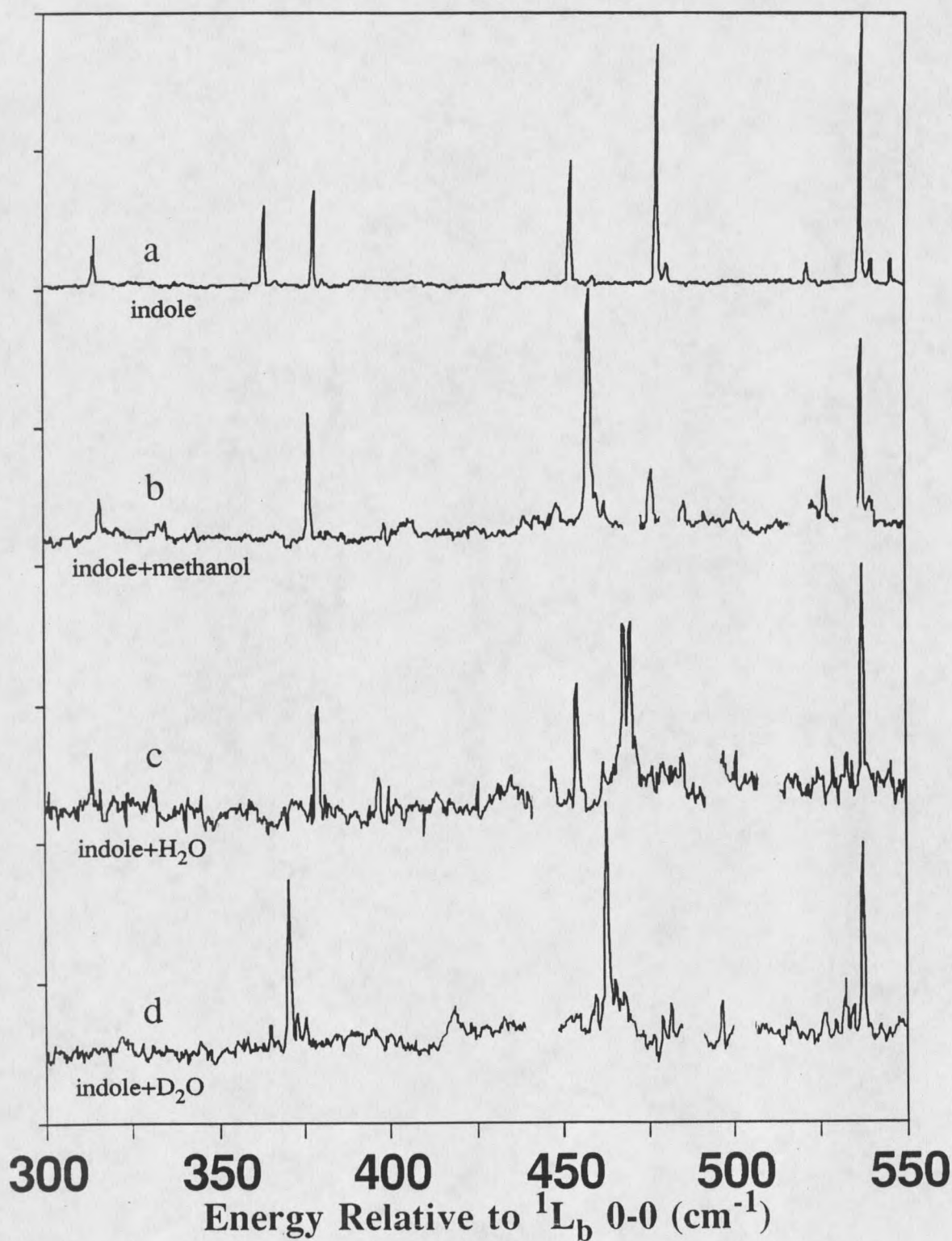


Figure 44. One-photon excitation spectra of the " 1L_a origin" region of indole, (a), indole+methanol, (b), indole+H₂O, (c), and indole+D₂O, (d), in supersonic jet. The last three spectra have been shifted by 160, 132, and 132 cm^{-1} to the blue.

of indole+methanol, the transitions corresponding to intermolecular vibrational modes of the complexes have also been deleted.

The transitions assigned to the 1L_b state line up very well with their counterpart in bare indole, (there are some discrepancies in indole+D₂O due to the isotope effects). However, as pointed out previously, the 1L_a transitions shift very differently and can be assigned by visual inspection. This is not the only evidence to assign these transitions as the 1L_a origin of the complex. Figure 45a shows the two-photon fluorescence spectrum of the putative origin of indole+methanol. Although the signal to noise ratio is rather poor, the shape of the spectrum indicates that the transition has 1L_a nature. The polarization ratio is 0.7, although is not very accurate because of the noise.

Also in Figure 45, we have the two-photon excitation spectrum of the 26_0^1 level in indole+methanol. Its parent transition, at 718 cm^{-1} , has a polarization ratio of 1.2, (Sammeth¹²⁷ reports a value of 1.3). The experimental Ω for the transition in the complex is 1.0, which may indicate that complexation alters the nature of the two-photon tensor.

Before finishing this discussion, the problem of the smaller than expected red shift for the 1L_a transitions must be addressed. Not only do calculations predict that the shift should be several times larger than for 1L_b , but also experimental results on the absorption red shift in bulk solution¹³ reflect this behavior of the 1L_a state with respect to 1L_b .

One way to explain this phenomenon is by invoking an avoided crossing between the 1L_a and 1L_b states. Our calculations show that when both states become nearly

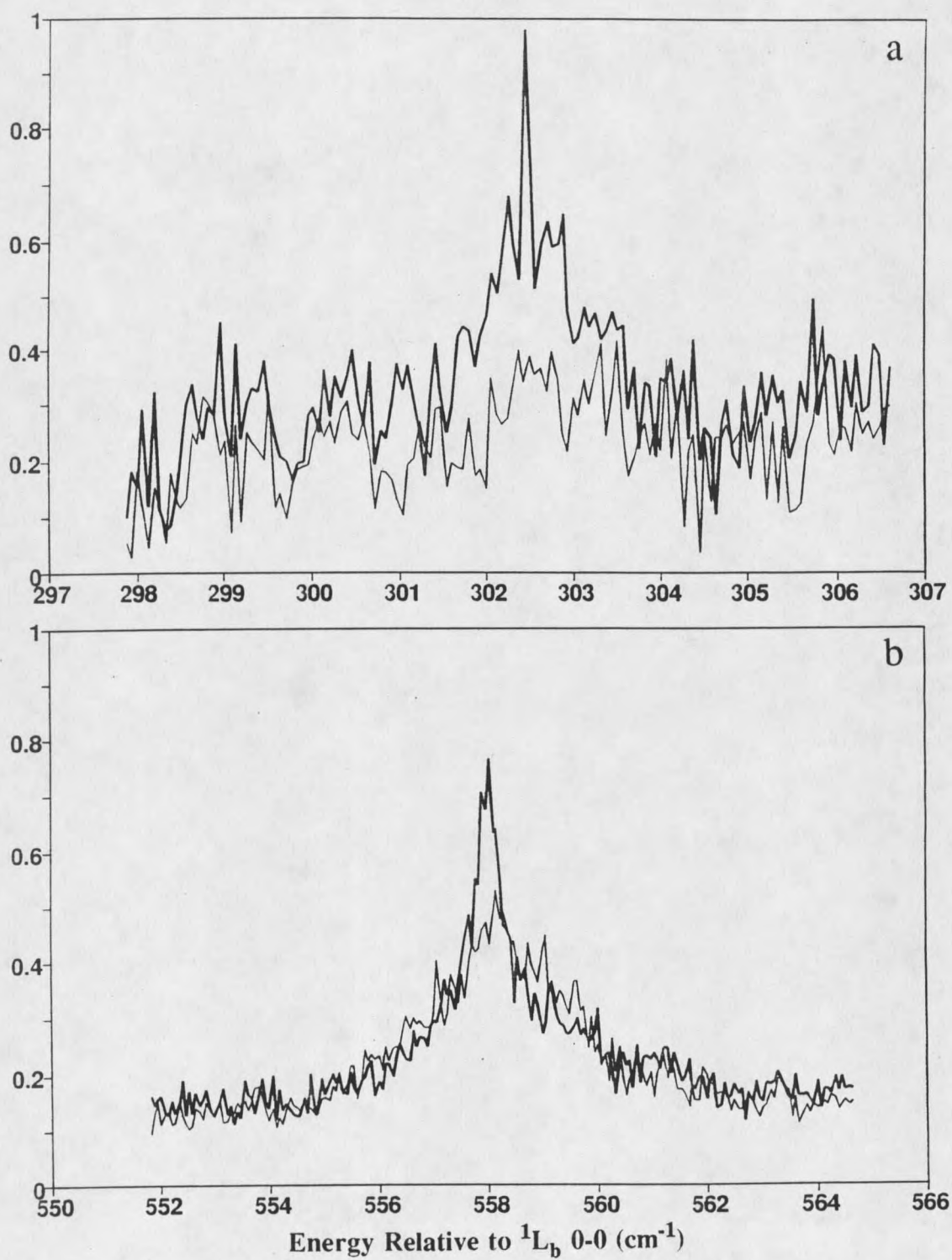


Figure 45. Two-photon excitation spectra of the 1L_a origin, (a), and the 26_0^1 mode, (b), of indole+methanol in supersonic jet.

degenerate, they mix, pushing the resulting states apart. The distance between the observed states is around $400\text{-}500\text{ cm}^{-1}$, which is the spacing observed in the spectra of indole and indole+solvent. The presumed avoided crossing would explain the relative displacement of the peaks, but it would also predict that the nature of the peaks corresponding to the complexes would be reversed with respect to the peaks in the bare molecule. That is, the origin of complex I should have 1L_a character and the peak at 341 cm^{-1} to the blue of the origin, (in indole+ H_2O), should have 1L_b character. Therefore, our two-photon data can be used to rule out the hypothesis of an avoided crossing between the two states to explain the shifts in the spectra of the complexes.

It is possible that the minimum of the indole-solvent dimer is not a conformation that favors the 1L_a red shift. In our calculations we showed, (see Figure 13), that only specific positions around the ring have the net effect of stabilizing significantly the 1L_a state. Those positions were believed to be the ones most likely to help the charge transfer associated to the excitation to the 1L_a state. If the solvent molecule hydrogen-bonds to the N-H group, with the oxygen in the plane of the indole ring, (as suggested in our calculations), its dipole would be roughly perpendicular to that of the indole molecule. In this case, not much differential effect between the two states would be induced. We have performed some calculations to test this hypothesis, and the results are somewhat encouraging. To this effect, we generated multiple geometries for an indole+water dimer similar to that of complex I. We applied two restrictions to the system: the $\text{H1}_{\text{indole}}\text{-O}_{\text{water}}$ distance had to be $2.0\text{ \AA}$, and the oxygen had to remain in the plane of the ring. Then, we performed INDO/S over these geometries to get the transition energies.

In one case, where the water dipole formed a 50° angle with the N-H bond, (the molecule was on the side of the C9 atom), and the hydrogens were above and below the plane of the ring, INDO/S calculated a red shift of $\sim 100 \text{ cm}^{-1}$ for 1L_b and $\sim 150 \text{ cm}^{-1}$ for 1L_a , which still differs, but not as much, from experimental values.

Another possibility is for complex I to have a geometry like the one postulated for complex II, that is, with the solvent bonded to the π cloud. We have done calculations similar to these just reported in the previous paragraph with the solvent molecule over the indole ring. Multiple geometries were generated again, varying its position over the ring and the angle between the water dipole and the plane of the indole molecule. The results were more dramatic in this case. One particular conformation, with the water above C9, bridging over both rings, and with its dipole forming a 40° angle with respect to the molecular plane, resulted in a calculated red shift of $\sim 70 \text{ cm}^{-1}$ for both states.

This last result, couples with calculations shown previously in this section suggesting that a hydrogen-bonded complex should be responsible for a larger red shift than one bonded to the π cloud, might indicate that the geometry of complex I is different from what was believed in the literature. Certainly, suggestions based on computer models are not reliable enough to overthrow an existing model. However, it must also be pointed out that the only experimental evidence on the geometry of the complexes consists of a linear relation found by Hager and Wallace between¹⁵³ between red shift and proton affinity. This relation was found for hydrogen-bond accepting solvents, and it was presumed that the solvents were bound to H1. Tubergen and Levy²⁶ found that the red shift induced by Complex I falls in the *experimental* curve obtained using this linear relation.

SUMMARY AND CONCLUSIONS

From our calculations and our experimental results, we have learned about the influence of the solvent on the energy levels of the indole chromophore.

Theoretical Methods

The modeling of the solute-solvent interactions is very sensitive to the dipole of the excited state. In fact, there is a *quadratic* relation between the fluorescence red shift and a function of the permanent dipole of the ground and excited states, (see Figure 24). However, there seems to be no relation between the red shift and the dipole of the solvent, possibly because the orientational polarizability of the solvent is proportional to a function of the dielectric which is nearly constant when $\epsilon > 10$. The changes in the solvent charges that we used were not large enough to bring the dielectric constant below this value. The use of different molecular dynamics programs or different shapes for the solvent cage does not seem to affect the calculated red shift, either.

The solvent relaxation, (after excitation of the solute to the 1L_a state), shows two distinctive types of decay. The fast component is inertial in character and has a Gaussian shape. The correlation time for this component goes from ~ 15 fs (for water) to > 300 fs, (for DME). We have shown that this fast response is associated with rotational motion of the solvent molecules in order to realign their dipoles in a way that aids the electron transfer which is characteristic of excitation to the 1L_a state. The slow component of the

solvent reorientation, with correlation times on the order of 200 fs to 10 ps, depending on the solvent, is related to the translational properties of the solvent, and responsible for a red shift 50-100% larger than that corresponding to the fast component.

From our calculations in butanol at 0 K, we have found that even if the solvent is rigid, we can expect a fast, (1-5 ps), red shift in the fluorescence, as large as 1000-2000 cm^{-1} . This red shift, as in the simulations at 300 K, comes from the inertial response of the solvent molecules to aid in the electron transfer in the indole ring. The slow response seen at 300 K is obviously not seen here, since the solvent molecules have no kinetic energy. However, it is possible that some of the excitation energy is transferred to the neighboring solvent molecules, (via coupling to the rotational inertial displacements), causing a local melting of the solution, and rearrangement into a new minimum.

There is a significant difference in the calculated red shift depending on whether the polarizability of the solute is included or not. This difference arises from the large effect of the dipole on the shift, (see above). Inclusion of polarizability has the effect of increasing the solvent field inducing dipole by as much as 50%.

Although the predicted fluorescence red shifts agree very well with experimental values, the results are rather poor for the calculated absorption shifts. When the ground state dipole is small, the orientational polarizability of the solvent is smaller than the electronic polarizability, which is neglected in our calculations. As a result, we are neglecting a contribution as large as, (or larger than), the one we are including, and the calculated shifts are significantly smaller, (50-70%), than the experimental value.

As a response to the large solvent field, the 1L_a state increases its charge transfer

character. This can be seen in the reduction of oscillator strength from ~ 0.30 to ~ 0.15 . The permanent dipole moment of the state increases by a factor of $\sim 60\%$.

Differences in free energies of solvation between the ground and excited states can be obtained from spectroscopic data. The values obtained from our calculated shifts are in good agreement with those obtained from experimental values.

Calculations done on non-zwitterionic tryptophan in the gas phase suggest that a large number, (>60), of conformers are possible, although only 12 of them are significantly populated at 300 K. This has an important consequence in the spectrum of tryptophan, since each of these conformations is going to have a distinctive origin and a progression similar to that observed for indole, resulting in a more complicated spectrum.

Experimental

From our fluorescence excitation spectra of supersonic expansions of indole in the presence of solvents, we have identified two types of complexes for the systems indole+H₂O, indole+D₂O, (with several isotopic mixtures), and indole+methanol. In the first case, we have identified an intermolecular vibrational mode of complex I, (see below), and three for complex II. For the indole+methanol system, we have identified one intermolecular vibrational mode for complex I, and seven for complex II.

The two-photon excitation spectrum of the origin of complex I for indole+methanol shows a polarization ratio of 1.36 and a shape similar to that of the spectrum of the origin. This is consistent with the expected 1L_b character of the transition. Attempts to take a two-photon spectrum of the origin of complex II were

unsuccessful.

Complex I, (for the three solvents studied), presents several ring-vibrational modes. The shifts of these transitions with respect to their counterparts in the bare molecule spectrum are similar to the spacing between the origin of complex I and the 1L_b origin of the bare molecule. For the in-plane 1L_b modes, the shifts are identical within experimental error. For out-of-plane 1L_b modes, the shifts are approximately 5 cm^{-1} less than the reference.

Ring-vibrational modes of complex II were observed, indicating that the origin of the complex must be 1L_b in nature. If there was only one complex, and what we labelled as complex II was just the 1L_a origin of such complex, it would be an exceptional coincidence that some ring-vibrational modes associated to the 1L_a state had the same frequencies as their counterparts in the 1L_b state.

Only one ring-vibrational mode of complex I was observed above 1000 cm^{-1} . This may suggest that the excitation energy causes dissociation of the complex. On the other hand, it is possible that more transitions are just hidden in this congested region of the spectrum.

We have calculated, (from spectroscopic data and using molecular mechanics), the relative displacement of the minima of the potential wells of the ground and excited states of complexes I and II for indole+water. These calculations, along with measurements of the relative intensities of the trimers, (indole+H₂O+He), formed in both complexes, suggest that complex I has the solvent hydrogen bonded to the N-H group and complex II has the solvent bonded to the π cloud. Calculations using MOPAC and molecular

mechanics have also found that the conformations just mentioned correspond to minima of the potential energy surface. No other minima have been found using these two methods.

A potential energy curve for the excited state of complex II has been studied by analysis of the fundamental and 6 overtones of the intermolecular vibration labelled ν_1 of the complex indole+H₂O. The differential spacings resulted in a second order anharmonicity constant, χ'_e , equal to 0.0076 ± 0.0006 , and a third order constant, y'_e , equal to 0.0000 ± 0.0001 . These results seem to indicate that the surface is fairly harmonic for the first 7 vibrational levels.

The hydrogen at position 1 in indole is exchanged for deuterium in the presence of D₂O. This complicates the spectrum of the system, since several isotopic species coexist and form, at least, three different types of complexes. Other types may be present, adding broadness to the peaks. The origin of complex II of *d*-indole+D₂O is more red shifted than the origin of the complex in *h*-indole+H₂O. This is expected if one assumes that the intermolecular modes are higher in frequency in the excited states. On the other hand, the intermolecular vibrational levels of both complexes, (I and II), in both isotopic species are different, as expected. Their relative frequencies agree with the notion that the whole water molecule is moving, (and not just the hydrogens).

The complexes between indole and water or methanol do not show the transition labelled 41_0^2 in the spectrum of indole. This absence cannot be attributed to lack of intensity, since there are other transitions, like 42_0^2 in both complexes, which would be expected to have less intensity than 41_0^2 and are seen. It can be postulated that the

position of the solvent in complex I is such that it disrupts mode 41 so much that it is no longer present or it appears at a completely different wavelength.

Conclusions

Solvation in water brings the 1L_a and 1L_b states closer together. Even in the ground state, where the geometry of the ring is far away from the 1L_a minimum, there are some solvent conformations that bring the 1L_a vertical transition energy below that of 1L_b . This seems to indicate that the 1L_a origin, which is at least 3000 cm^{-1} lower than the vertical transition energy, (from experimental spectra), is below the 1L_b origin in water solution.

There is no need to invoke exciplex formation to explain the red shift in fluorescence, since it can be well reproduced by regular electrostatic interactions. The mechanism of the fast response of solvent to solute excitation has been found to correspond to rapid realignment of the solvent dipole, (by motion of the hydroxyl hydrogens). Only the solvent molecules within $\sim 6\text{ \AA}$ of the solute feel its change in dipole. This is not surprising considering that the torque felt by the solvent dipole is inversely proportional to the cube of the distance to the solute. Once the solvent has relaxed, there is evidence of hydrogen-bond formation around certain atoms, like C4, which have been recipients of a large amount of electron transfer.

The method presented here includes, (as shown in the previous paragraph), the solvent motion required to explain spectroscopic shifts. Therefore, this method is useful for predicting the differential behavior of a fluorescing molecule in different solvents.

In this regard, this method is qualitatively superior to other methods which rely solely on the dielectric properties of the solvent, because the Onsager-Mataga-Lippert formulation is not sensitive to changes in the dielectric constant when its value is above 10, which is the case in polar solvents

Two-photon excitation studies of the putative 1L_a origin of the indole+methanol complex show a polarization ratio and shapes of the spectra under different polarizations compatible with 1L_a character. However, the shifts associated with the "480" 1L_a origin of the complex are always larger than the reference shift, but not much larger, (between 10 and 15 cm^{-1} , depending on the solvent). This agrees with calculated values, (and experimental values in bulk solution), indicating that 1L_a states should shift more than 1L_b states in the presence of a polar solvent; but disagrees with these same calculations because the differential shift is not large enough. The shifts associated with the "455" 1L_a origin always shift less than the reference shift. There is also a large variation in the relative intensities of these two transitions in the different systems. The fact that the " 1L_a " shifts are not that much different from those seen in the 1L_b states may lead one to invoke the existence of an avoided crossing between these two states, which would result in a similar spacing between their origins both in the bare molecule spectrum and in the spectra in solution. However, under these circumstances, the nature of the origins should be reversed upon complexation, that is, the origin of the complex should have 1L_a character and the peak $\sim 480 \text{ cm}^{-1}$ to the blue should be 1L_b in nature. As two-photon spectroscopy indicates that this is not the case, the evidence presented here suggests that "455" and "480" are 1L_b transitions that have borrowed some 1L_a character.

REFERENCES CITED

1. J. M. Hollas, *Spectrochim. Acta* 19, 753 (1963).
2. M. S. Walker, T. W. Bednar, and R. Lumry, *J. Chem. Phys.* 47, 1020 (1967).
3. J. Eisinger and G. Navon, *J. Chem. Phys.* 50, 2069 (1969).
4. E. H. Strickland, J. Horwitz, and C. Billups, *Biochemistry* 9, 4914 (1970).
5. M. S. Walker, T. W. Bednar, R. Lumry, and F. Humpries, *Photochem. Photobiol.* 14, 147 (1971).
6. B. Valeur and G. Weber, *Photochem. Photobiol.* 25, 441 (1977).
7. A. Lautié, M. F. Lautié, A. Gruger, and S. A. Fakhri, *Spectrochim. Acta* 36A, 85 (1980).
8. E. H. Strickland, C. Billups, and E. Kay, *Biochemistry* 11, 3657 (1972).
9. E. H. Strickland and C. Billups, *Biopolymers* 12, 1989 (1973).
10. M. R. Eftink, and L. A. Selvidge, P. R. Callis, and A. A. Rehms, *J. Phys. Chem.* 94, 3469 (1990).
11. G. Webber, *Biochem. J.* 75, 335 (1959).
12. J. R. Platt, *J. Chem. Phys.* 17, 484 (1949).
13. H. Lami and N. Glasser, *J. Chem. Phys.* 84, 597 (1986).
14. S. V. Konev, Fluorescence and Phosphorescence of Proteins and Nucleic Acids, (Plenum, New York, 1967).
15. M. Martinaud and A. Kadiri, *Chem. Phys.* 28, 473 (1978).
16. L. J. Andrews and L. S. Forster, *Biochemistry*, 11, 1875 (1972).
17. J. W. Longworth, in Excited States of Proteins and Nucleic Acids, edited by R. F. Steiner and I. Weinryb (Plenum, New York, 1971), p.319.
18. J. R. Beechem and L. Brand, *Ann. Rev. Biochem.* 54, 43 (1985).
19. J. R. Lakowicz, Principles of Fluorescence Spectroscopy, (Plenum Press, New York, 1983).

20. A. P. Demchenko, Ultraviolet Spectroscopy of Proteins, (Springer-Verlag, Berlin, 1986).
21. H. Abe, N. Mikami, and M. Ito, *J. Phys. Chem.* **86**, 1768 (1982).
22. J. Hager and S. C. Wallace, *J. Phys. Chem.* **87**, 2121 (1983).
23. T. Montoro, C. Jouvét, A. López-Campillo, and B. Soep, *J. Phys. Chem.* **87**, 3582 (1983).
24. R. Bersohn, U. Even, and J. Jortner, *J. Chem. Phys.* **80**, 1050 (1984).
25. J. Hager, M. Ivanko, M. A. Smith, and S. C. Wallace, *Chem. Phys.* **105**, 397 (1986).
26. M. J. Tubergen and D. H. Levy, *J. Phys. Chem.* **95**, 2175 (1991).
27. G. A. Bickel, D. R. Demmer, E. A. Outhouse, and S. C. Wallace, *J. Chem. Phys.* **91**, 6013 (1989).
28. T. R. Rizzo and D. H. Levy, Lasers and Mass Spectroscopy, Chapter 18, edited by D. M. Lubman, (Oxford University Press, New York, 1990).
29. D. R. Demmer, G. W. Leach, E. A. Outhouse, J. W. Hager, and S. C. Wallace, *J. Chem. Phys.* **94**, 582 (1990).
30. S. Arnold and M. Sulkes, *J. Phys. Chem.* **96**, 4768 (1992).
31. A. A. Rehms, Ph. D. Thesis, Montana State University, 1990.
32. B. E. Anderson, R. D. Jones, A. A. Rehms, P. Ilich, and P. R. Callis, *Chem. Phys. Lett.* **125**, 106 (1986).
33. A. A. Rehms, and P. R. Callis, *Chem. Phys. Lett.* **140**, 83 (1987).
34. J. R. Reimers, N. S. Hush, D. M. Sammeth, and P. R. Callis, *Chem. Phys. Lett.* **169**, 622 (1990).
35. D. M. Sammeth, S. Yan, L. H. Spangler, and P. R. Callis, *J. Phys. Chem.* **94**, 7340 (1990).
36. R. A. Marcus, *J. Chem. Phys.* **24**, 966 (1956).
37. R. A. Marcus and N. Sutin, *Biochem. Biophys. Acta* **811**, 265 (1985).

38. J. Ulstrup, Charge Transfer Processes in Condensed Media, (Springer, New York, 1979).
39. A. J. Ruggiero, D. C. Todd, and G. R. Fleming, *J. Am. Chem. Soc.* **112**, 1003 (1990).
40. M. Cho, S. J. Rosenthal, N. F. Scherer, L. D. Ziegler, and G. R. Fleming, *J. Chem. Phys.* **96**, 5033 (1992).
41. S. J. Rosenthal, X. Xie, M. Du, and G. R. Fleming, *J. Chem. Phys.* **95**, 4715 (1991).
42. P. F. Barbara and W. Jarzęba, *Adv. Photochem.* **15**, 1 (1990).
43. W. Jarzęba, G. C. Walker, A. E. Johnson, M. A. Kahlow, and P. F. Barbara, *J. Phys. Chem.* **92**, 7039 (1988).
44. R. Jiménez and G. R. Fleming, private communication.
45. M. Maroncelli and G. R. Fleming, *J. Chem. Phys.* **89**, 5044 (1988).
46. M. Maroncelli, *J. Chem. Phys.* **94**, 2084 (1991).
47. R. M. Levy, D. B. Kitchen, J. T. Blair, and K. Krogh-Jespersen, *J. Phys. Chem.* **94**, 4470 (1990).
48. L. Perera and M. L. Berkowitz, *J. Chem. Phys.* **96**, 3092 (1992).
49. E. Neria and A. Nitzan, *J. Chem. Phys.* **96**, 5433 (1992).
50. P. L. Muiño, D. Harris, J. Berryhill, B. Hudson, and P. R. Callis, *Proceedings of the SPIE* **1640**, 240 (1992).
51. P. L. Muiño and P. R. Callis, submitted to *J. Chem. Phys.*
52. M. M. Karelson and M. C. Zerner, *J. Phys. Chem.* **96**, 6949 (1992).
53. C. F. Chabalowski, D. R. Garmer, J. O. Jensen, and M. Krauss, *J. Phys. Chem.* **97**, 4608 (1993).
54. M. W. Wong, K. B. Wiberg, and M. J. Frisch, *J. Chem. Phys.* **95**, 8991 (1991).
55. C. J. Cramer and D. G. Truhlar, *J. Am. Chem. Soc.* **113**, 8305 (1991).

56. J. P. Lowe, Quantum Chemistry, (Academic Press, Orlando, 1978)
57. J. C. Slater, *Phys. Rev.* **36**, 57 (1930).
58. A. Veillard and E. Clementi, *J. Chem. Phys.* **49**, 2415 (1968).
59. P. R. Callis, *J. Chem. Phys.* **95**, 4230 (1991).
60. E. Clementi and J. Mehl, *Jerusalem Symp. Quantum Chem. Biochem.* **6**, 137 (1974).
61. J. A. Pople, D. P. Santry and G. A. Segal, *J. Chem. Phys.* **43**, S129 (1965).
62. J. A. Pople and G. A. Segal, *J. Chem. Phys.* **43**, S136 (1965).
63. J. A. Pople and G. A. Segal, *J. Chem. Phys.* **44**, 3289 (1966).
64. D. P. Santry and G. A. Segal, *J. Chem. Phys.* **47**, 158 (1967).
65. D. P. Santry, *J. Am. Chem. Soc.* **90**, 3309 (1968).
66. J. Ridley and M. Zerner, *Theor. Chim. Acta (Berl.)* **32**, 111 (1973).
67. J. Ridley and M. Zerner, *Theor. Chim. Acta (Berl.)* **42**, 223 (1976).
68. A. Bacon and M. Zerner, *Theor. Chim. Acta (Berl.)* **53**, 21 (1979).
69. M. C. Zerner, G. H. Loew, R. F. Kirchner, and U. T. Mueller-Westerhoff, *J. Am. Chem. Soc.* **102**, 589 (1980).
70. P. R. Callis, *Photochem. Photobiol.* **44**, 315 (1986).
71. D. Theiste, R. D. Jones, and P. R. Callis, *Chem. Phys. Lett.* **133**, 14 (1987).
72. D. Theiste, P. R. Callis, and R. W. Woody, *J. Am. Chem. Soc.* **113**, 3260 (1991).
73. N. Mataga and K. Nishimoto, *Z. Phys. Chem. (Frankfurt)* **13**, 140 (1955).
74. G. Klopman, *J. Am. Chem. Soc.* **86**, 4550 (1964).
75. R. Pariser and R. G. Parr, *J. Chem. Phys.* **21**, 767 (1953).
76. W. D. Edwards, N. Y. Orhn, B. L. Weiner, and M. C. Zerner, *Int. J. Quantum Chem. Symp.* **18**, 507 (1984).

77. W. A. Parkinson and M. C. Zerner, *J. Chem. Phys.* **94**, 478 (1991).
78. R. L. Ellis, G. Kuehnlenz, and H. H. Jaffé, *Theoret. Chim. Acta (Berl.)* **26**, 131 (1972).
79. P. Dauber-Osguthorpe, V. A. Roberts, D. J. Osguthorpe, J. Wolff, M. Genest, and A. T. Hagler, *Proteins: Struct. Func. Gen.* **4**, 31 (1988).
80. S. J. Weiner, P. A. Kollman, D. A. Case, U.C. Singh, C. Ghio, G. Alagona, S. Profeta, Jr., and P. Weiner, *J. Amer. Chem. Soc.* **106**, 765 (1984).
81. S. J. Weiner, P. A. Kollman, D. T. Nguyen, and D. A. Case, *J. Comp. Chem.* **7**, 230 (1986).
82. R. Fletcher, Practical Methods of Optimization, vol. 1, (John Wiley and Sons, New York, 1980).
83. H. J. C Berendsen, J. P. M. Postma, W. F. Van Gunteren, A. DiNola, and J. R. Haak, *J. Chem. Phys.* **81**, 3684 (1984).
84. L. Onsager, *J. Am. Chem. Soc.* **58**, 1486 (1936).
85. P. Debye, *Physik. Z.* **13**, 97 (1912).
86. M. W. Wong, M. J. Frisch, and K. B. Wiberg, *J. Am. Chem. Soc.* **113**, 4776 (1991).
87. Y. Ooshika, *J. Phys. Soc. Japan* **9**, 594 (1954).
88. N. Mataga, Y. Kaifu, and M. Koizumi, *Bull. Chem. Soc. Japan*, **28**, 690 (1955).
89. N. Mataga, Y. Kaifu, and M. Koizumi, *Bull. Chem. Soc. Japan*, **29**, 465 (1956).
90. E. Lippert, *Z. Naturforsch* **10a**, 541 (1955).
91. E. Lippert, *Z. Physik. Chem. (Frankfurt)* **6**, 125 (1956).
92. E. G. McRae, *J. Phys. Chem.* **61**, 562 (1957).
93. E. G. McRae, *Spectrochim. Acta* **12**, 192 (1958).
94. R. A. Marcus, *J. Chem. Phys.* **38**, 1335 (1963).
95. R. A. Marcus, *J. Chem. Phys.* **39**, 1734 (1963).

96. R. A. Marcus, *J. Chem. Phys.* **43**, 1261 (1965).
97. R. A. Marcus, *J. Chem. Phys.* **38**, 1858 (1963).
98. N. G. Bakhsiev, Y. T. Mazurenko, and I. V. Piterskaya, *Opt. Spektrosk.* **21**, 550 (1966).
99. J. Birks, Photophysics of Aromatic Molecules, (Wiley, New York, 1970).
100. R. M. Levy, J. D. Westbrook, D. B. Kitchen, and K. Krogh-Jespersen, *J. Phys. Chem.* **95**, 6756, (1991).
101. F. H. Stillinger and A. Rahman, *J. Chem. Phys.* **57**, 1281 (1972).
102. F. H. Stillinger and A. Rahman, *J. Chem. Phys.* **60**, 1545 (1974).
103. R. O. Watts, *Chem. Phys.* **26**, 367 (1977).
104. H. J. C Berendsen, J. P. M. Postma, W. F. Van Gunteren, and J. Hermans, Intermolecular Forces, edited by B. Pullman, p. 331 (1981).
105. W. L. Jorgensen, *Chem. Phys. Lett.* **92**, 405 (1982).
106. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein, *J. Chem. Phys.* **79**, 926 (1983).
107. C. L. Brooks, B. M. Pettitt, and J. Karplus, *J. Chem. Phys.* **83**, 5897 (1985).
108. K. Lau, private communication.
109. M. Thompson, private communication.
110. J. I. Steinfeld, Molecules and Radiation, (MIT Press, Cambridge, 1986).
111. D. M. Sammeth, S. S. Siewert, P. R. Callis, and L. H. Spangler, *J. Phys. Chem.* **96**, 5771 (1992).
112. D. M. Sammeth, S. S. Siewert, L. H. Spangler, and P. R. Callis, *Chem. Phys. Lett.* **193**, 532 (1992).
113. R. E. Smalley, L. Wharton, and D. H. Levy, *J. Chem. Phys.* **63**, 4977 (1975).
114. M. Göppert-Mayer, *Ann. Rev* **9**, 273 (1929).

115. W. M. McClain, *J. Chem. Phys.* **57**, 2264 (1972).
116. W. M. McClain, *Acc. Chem. Res.* **7**, 129 (1974).
117. W. M. McClain and R. A. Harris, Excited States, 1, E. C. Lim Editor, (Academic Press, New York, 1977).
118. F. Metz, W. E. Howard, L. Wunsch, H. J. Neusser, and E. W. Schlag, *Proc. R. Soc. Lond. A* **363** 381 (1978).
119. P. R. Monson, W. M. McClain, *J. Chem. Phys.* **53**, 29 (1970).
120. P. R. Callis, *J. Chem. Phys.* **99**, 27 (1993).
121. D.-H. Lee, Ph. D. Thesis, Cornell University, 1983.
122. T. Takigawa, T. Ashida, Y. Sasada, and M. Kakudo, *Bull. Chem. Soc. Jpn.* **39**, 2369 (1966).
123. J. T. Vivian, Master's Thesis, Montana State university, 1993.
124. B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus, *J. Comp. Chem.* **4**, 187 (1983).
125. A. Chandra and B. Bagchi, *Chem. Phys. Lett.* **165**, 93 (1990).
126. S. A. Williams, Ph. D. Thesis, Montana State University, 1989.
127. D. M. Sammeth, Ph. D. Thesis, Montana State University, 1992.
128. B. Anderson, Ph. D. Thesis, Montana State University, 1987.
129. B. Dick, H. Gonska, and G. Hohlneicher, *Ber. Bunsenges. Phys. Chem.* **85**, 746 (1981).
130. R. D. Jones and P. R. Callis, *J. Appl. Phys.* **64**, 4301 (1988).
131. S. R. Meech, D. Phillips, and A. G. Lee, *Chem. Phys.* **80**, 317 (1983).
132. G. J. Smith and W. H. Melhuish, *J. Phys. Chem.* **95**, 4288 (1990).
133. T. Montoro, M. Chabbert, J. Tyrzyk, and H. Lami, *J. Chem. Phys.* **89**, 2712 (1988).

134. B. Bagchi and A. Chandra, *J. Chem. Phys.* **97**, 5126 (1992).
135. S. C. Wallwork, *Acta Cryst.* **15**, 758 (1962).
136. S. N. Vinogradov and R. H. Linnell, Hydrogen Bonding, (Van Nostrand Reinhold Co., New York, 1971).
137. A. J. Gotch and T. S. Zwier, *J. Chem. Phys.* **96**, 3388 (1992).
138. Rothschild, Dynamics of Molecular Liquids, (Wiley, New York, 1984).
139. A. P. Demchenko and A. S. Ladokhin, *Eur. Biophys. J.* **15**, 369 (1988).
140. H. L. Gordon, H. C. Jarrell, A. G. Szabo, K. J. Willis, and R. L. Somorjai, *J. Phys. Chem.* **96**, 1915 (1992).
141. H. E. Alper and R. M. Levy, *J. Chem. Phys.* **91**, 1242 (1989).
142. M. Newmann, *J. Chem. Phys.* **85**, 1567 (1986).
143. H. Gordon and S. Goldman, *Mol. Simul.* **2**, 177 (1989).
144. I. Gonzalo and J. L. Escudero, *J. Phys. Chem.* **86**, 2896 (1982).
145. O. A. Karim, A. D. J. Haymet, M. J. Banet, and J. D. Simon, *J. Phys. Chem.* **92**, 3391 (1988).
146. E. W. Castner, Jr., B. Bagchi, M. Maroncelli, S. P. Webb, A. Ruggiero, and G. R. Fleming, *Ber. Bunsenges. Phys. Chem.* **92**, 363 (1988).
147. T. R. Rizzo, Y. D. Park, and D. H. Levy, *J. Chem. Phys.* **85**, 6945 (1986).
148. M. J. Tubergen, J. R. Cable, and D. H. Levy, *J. Chem. Phys.* **92**, 51 (1989).
149. E. A. Outhouse, G. A. Bickel, D. R. Demmer, and S. C. Wallace, *J. Chem. Phys.* **95**, 6261 (1991).
150. S. H. Lim, Y. Fujimura, H. J. Neusser, and E. W. Schlag, Multiphoton Spectroscopy of Molecules, (Academic Press, New York, 1984).
151. J. W. Hager and S. C. Wallace, Lasers and Mass Spectroscopy, Chapter 19, Edited by D. M. Lubman, (Oxford University Press, New York, 1990).
152. Y. Nibu, H. Abe, N. Mikami, and M. Ito, *J. Phys. Chem.* **87**, 3898 (1983).

- 153. J. Hager and S. C. Wallace, *J. Phys. Chem.* 88, 5513 (1984).
- 154. J. Franck, *Trans. Faraday Soc.* 21, 536 (1925).
- 155. E. U. Condon, *Phys. Rev.* 32, 858 (1928).
- 156. R. L. Fulton and M. Gouterman, *J. Chem. Phys.* 41, 2280 (1964).
- 157. J. D. Graybeal, Molecular Spectroscopy, (McGraw-Hill, New York, 1988).

APPENDICES

APPENDIX A

Files Used in the Theoretical Methods

The programs and command files necessary to run the calculations described in this thesis are stored in the subdirectory *Fortran* in the account *muino* of the Personal Iris. They are also stored on tape. A subdirectory of *Fortran*, named *files*, contains other files needed to execute these programs.

Command Files

Two types of command files are necessary to perform these calculations. They are generally labelled *Run* and *Rup*.

Run is used for the simulations where we obtain information about absorption. The version stored in the subdirectory *Fortran* is designed to run the system for 5 ps, stopping every 5 fs and calculating the transition energies for both 1L_a and 1L_b states. The input files needed are the following:

1. A cartesian coordinate file, (*.car*).
2. A molecular data file, (*.mdf*).
3. A molecular dynamics restart file, (*.ors*).
4. A molecular dynamics input file, (*.inp*).

The last of these files has to be set to run for 5 fs and write an archive file, (*.arc*), at the end of the 5 fs. (Normally this file is named *5.inp*). It also requires some of the

fortran programs that will be described below).

The *Rup* command file is required to run the fluorescence calculations. It runs molecular dynamics continuously for 5000 fs, in order to generate a starting point reasonably far from the previous calculation. Then, it runs for 500 fs in the ground state, stopping every 10 fs, in order to calculate the transition energies; for 200 fs in the 1L_a state, (stopping every 5 fs); and, finally, for 3.8 ps in the 1L_a state, (stopping every 10 fs). The same type of files as before are needed, plus two extra molecular dynamics input files: one set to run for 10 fs, (*10.inp*), and another set to run for 5000 fs, (*5000.inp*).

To adapt either of these files to other systems, (like 3MI in butanol, indole in water, ...), the only necessary changes in the files are:

1. Change the directory where Discover is to be run.
2. Change the root of the filenames.
3. Change the **.inp* files so that they run for the required amount of time, (see METHODS section).
4. Change the root of the output files.

The command files are set to produce the following output:

1. Restart files, (*.ors*), so a new calculation can be started where the last finished.

Rup provides also three intermediate restart files: the first is generated after the 5000 fs run, the second is created at the point where the molecule is excited, and the third is generated at the point where molecular dynamics stops running at 5 fs intervals.

2. Archive files, (*.arc*), which are like cartesian coordinates files, (*.car*), but have a different format and use less space.

3. Summary files, (*.sum*), from INDO/S.
4. Transition Density files, (*.tds*), also from INDO/S.
5. One file containing the charges of the solute in the ground state for every INDO/S calculation, and, in *Rup*, another file containing the excited state charges.

Run generates 1000 files of types 2, 3, and 4. *Rup* generates 470.

Programs

The following is a list of the programs used. All of them, (unless stated otherwise), were written for a system consisting of 3MI and 252 water molecules, but sometimes there are versions of these programs prepared for other systems. For instance, *com.f* generates an input file for INDO/S from a cartesian coordinate file of 3MI in water; *icom.f* and *tcom.f* do the same for *indole* and *tryptophan* in water; *comb.f*, *comm.f*, and *comd.f* are similar, but for 3MI in *butanol*, *methanol*, and *dimethylether*, respectively.

It is possible that some of these programs need to be slightly modified if the number of solvent molecules is changed, or a new version of Discover, (with different sized headings in its files), is used.

autocorrelation.f is a program that calculates the autocorrelation function of a numerical function. It reads free format files with two columns of data: the first is time and the second is a function of time.

car.f reads a list of charges, (prepared with *charges.f* or *f06.f*, see below), and prepares part of a cartesian coordinate file, (*.car*). The heading, (*car.st*), and the coordinates of the solvent, (*car.end*), must be added to the output of *car.f*, (normally using *cat*, which is a unix command). To run *car.f*, a file labelled *lll.car*, containing the

coordinates and the atom types present in the molecule, is needed.

charges.f prepares the charges of the excited state from the charges of the ground state, (obtained with *f06.f*), and the difference in charge between excited and ground state, (from *tds.f*). The input file is an *f06.f* output file which has a *tds.f* output appended.

com.f reads the solvent coordinates from a cartesian coordinate file and prepares it to be used in the INDO/S input. It needs to be appended to a heading, (*startG.com* or *startLa.com*), containing the coordinates of the ground or excited states of the ring. Another file, *end.com*, containing molecular orbital information, needs to be appended to the *com.f* output.

f06.f reads and INDO/S output file, and obtains the charges for the ground state of the molecules.

getarc reads an archive file, (*.arc*), searches for a specific frame, (an archive file may contain many frames, each of which has the cartesian coordinates of 3MI with 252 water molecules, (or any other system), at a certain time in the calculation), and generates a cartesian coordinate file. The syntaxis to run this program is the following:

getarc filename.arc filename.car -i

where *i* is the frame number. This program works with any combination of solute and solvent, regardless of the number of solvent molecules.

mdf.f is equivalent to *car.f*, but prepares a molecular data file. The files used with it are *mdf.st*, *mdf.end*, and *lll.mdf*.

nentrvlf and *nentrvquattrolf* read two cartesian coordinates files and calculate the difference in translational, rotational, and vibrational contributions to the fluorescence red

shift between these two configurations. They only work for 3MI with 252 water molecules. The difference between these two programs is that the second produces output designed to be graphed using Quattro Pro, (from Borland).

sep.f reads a transition density file, (*.tds*), and decides which state of the system corresponds to 1L_a and which to 1L_b .

tds.f, (*tdsla.f* or *tdslb.f*), reads a transition density file and obtains the difference in charge between the 1L_a or 1L_b states and the ground state. The output is normally appended to the output file of *f06.f* in order to create an input file for *charges.f*.

zind is a command file to run INDO/S. It needs an input file, (*.com*), and generates three files: an output file, (*f06*), a summary file, (*.sum*), and a transition density file, (*.tds*). There are three programs, (*rsumm.f*, *rsumm1.f*, and *rsumm2.f*), which make different types of summary files. A program named *rtndl5.f* generates the *.tds* file. This program needs another input file, *tncl.in*, which specifies which density matrices are to be calculated. The programs to run INDO/S are stored in the subdirectory *z2* of the account *callis*. They are also stored in the same tape containing the command and fortran files specified in this appendix.

APPENDIX B

Experimental Setup and Procedures

This appendix contains information about the connections between the different electronic apparatuses needed to run experiments on one- and two-photon spectroscopy in the supersonic jet in Callis lab. It also contains information about the procedure to align the lenses and mirror in the two-photon experiments. Information on the electronics of the boxcar can be found in a manual written by S. Williams. A laboratory notebook labelled *Laser Notebook #1*, written by P. Muñio, describes most of the procedures required to operate and maintain the dye and Nd:YAG lasers. Finally, a manual labelled *Alignment of the HY-200 Nd:YAG Laser*, by P. Muñio, describes the procedure to realign this laser.

As stated in the METHODS section, the Iota Valve Controller drives the jet and the laser. A coaxial cable connects the outlet labelled *output*, (in the valve controller), to the external trigger of the Stanford Research delay. External Triggering and Delay A are chosen. The settings for Delay A are as follows: delay: ~600 μsec ; (it may change a few tens of μsec depending on the behavior of the nozzle); output: variable, (+1.9V, 50 Ω). The delayed pulse is taken from outlet A in the back panel of the delay box, (where all the voltages are multiplied by 10, so the actual amplitude of the pulse is +19 V), and directed to the outlet labelled *EXT* located inside the laser controller. (To make the connection, the cover of the controller must be removed and the laser turned off). The

knob labelled *Repetition Rate* in the laser must be set at *External*.

The amount of solvent in the line can be regulated using the metering valve located after the *solvent chamber*, (see Figure 8). For instance, the optimum intensity for the transitions corresponding to complex I in the indole+methanol system, (at 44°C in the sample chamber), is obtained when the valve reads 0.35.

Alignment of the Collecting Lenses and Mirror

To get signal in two-photon spectroscopy, the lenses must be carefully aligned. The process requires the use of the dye laser and a HeNe laser.

We define three orthogonal axes in the jet. The dye laser beam travels along the X axis, (see Figure 8); the fluorescence is collected along the Y axis; and, finally, the molecular beam travels along the Z axis. All these axes intersect in a point, with an error of less than 1 mm. It is important that the center of the lenses and mirror are in the Y axis, and that they focus the fluorescence along this axis.

To align the lenses and mirror, the following procedure can be followed.

1. Remove the lenses, mirror, mirror mount, PMT, filter holder, and the two windows along the Y axis.
2. Make two circular pieces of cardboard. Their diameters must be equal to the internal diameter of the openings in the jet along the Y axis. They must have a ~2 mm hole in the center. Place each cardboard piece in one of the jet openings.
3. Shine the dye laser, (at lowest power), along the X axis. Shine the HeNe along the Y axis. The hole in the cardboard pieces should help in the alignment.
4. At the bottom of the jet, place a lens holder supporting a vertical white wire, (as

narrow as possible). Move the wire until it partially blocks both dye and HeNe beams at the point where they cross.

5. Place the lenses in their guides. The position of the lenses along the Y axis should be roughly equal to what is expected, (based on their focal lengths), to yield the best collection. Juggle them until the HeNe beam exits the jet through the hole in the cardboard piece. (The HeNe beam will be expanded as a result of placing the lenses back in. One should pass the center of the expanded beam through the hole in the cardboard).

6. Put the mirror mount and the mirror back into the chamber. Adjust the mirror so the HeNe hits its center.

7. Alternatively blocking the HeNe and dye laser beams, a very careful adjustment must be done on the lenses. With the HeNe blocked, (and the lights off), the lenses must be moved along the Y axis to focus the reflection of the dye laser beam in the wire at the place where the PMT entrance is, (this must be estimated since the PMT is not in place). With the dye laser blocked, the lenses should be juggled to ensure that the HeNe beam passes through their center.

8. Alternatively blocking the HeNe and dye laser beams, the mirror positioning must be adjusted so that the reflection coming off the mirror superimposes the point made by focusing of the direct reflection from the wire. (The reflection from the mirror should be partially, (and, ideally, symmetrically), blocked by the wire).

9. Remove the wire and the cardboard pieces, and place the windows, filter holder and PMT back into the jet assembly.

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