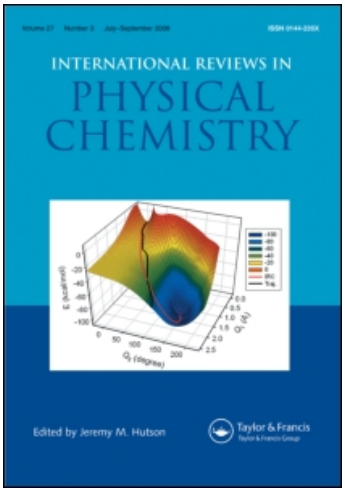

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Linear dichroism spectroscopy of gas phase biological molecules embedded in superfluid helium droplets

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Linear dichroism spectroscopy of gas phase biological molecules embedded in superfluid helium droplets

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This article presents the current status of gas phase linear dichroism (LD) spectroscopy, including the theoretical background, the experimental technique, and a few examples in the UV/VIS and IR. Orientation and alignment of gas phase samples are achieved using a DC electric field. To reach the necessary degree of alignment, biological molecules vaporized from a heated oven need to be embedded in superfluid helium droplets. Excitation under different polarization directions of the light source relative to the alignment field can then be used to derive the direction of the transition dipole, or the size of the permanent dipole, or both. For biological molecules that have no resonance lines or too many resonance lines, LD offers an additional parameter for spectroscopic assignment and tautomeric and conformational identification. The direction of the vibrational transition dipole is proven more reliable for vibrational and tautomeric assignment than the energy or frequency information, which is often problematic because of its sensitivity to basis sets and calculation methods. Several examples of vibrational LD of nucleic acid bases will be discussed. On the other hand, if a chromophore with a known electronic transition dipole is attached to a biological molecule, as demonstrated in the case of tryptamine, the permanent dipole determined from LD is then representative of the molecular conformation. This method of conformational determination does not rely on detailed spectroscopic assignment, thus it is applicable to molecules that do not have resolvable vibronic bands. However, its application is currently limited to the availability of an effective chromophore, and the search for such a chromophore is an on-going effort.

Keywords: polarization spectroscopy; linear dichroism; superfluid helium droplets; field induced orientation; molecular alignment

Contents

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1. Introduction

The field of gas phase spectroscopy of biological molecules has gone through several stages of metamorphosis over the last two decades [1–8]. The development of the laser desorption technique [9–12] and the fine tuning of the direct heating method [13,14] have pushed the limit of vaporization to small enzymes [15,16] and nucleic acid base pairs [17,18]. The ready availability of reliable infrared optical parametric oscillators (OPO) has accentuated the role of vibrational spectroscopy in conformational studies of flexible biological molecules [19–22]. The combination of rotational, vibrational, and electronic spectroscopy, achieved via a variety of laser techniques such as IR-UV and UV-UV hole burning and hole filling methods, has yielded tremendous information on intrinsic properties of biological molecules [7,23–27]. Polarization spectroscopy, including linear dichroism (LD) [28,29] and circular dichroism (CD) [30–34], has also been pursued at the most fundamental level [35,36] and has been applied for conformational analysis of gas phase samples [37,38]. Extensive work has been carried out on nucleic acid bases, nucleosides, nucleotides, base pairs, amino acids, small polypeptides, small enzymes, sugars, polysaccharides, and other 'small' biological molecules such as neural transmitters, in the frequency domain and the time domain [4,6,7,15,16,24,25,39–43]. Water complexes of these biological molecules mimicking the solution environment are also being actively investigated [44–48]. In addition to neutral molecules, ions have also gained quite some interest in recent years [49–53]. The success of electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) has revolutionized the field of mass spectrometry, particularly in its application in biology [54,55]. While the high sensitivity of mass spectrometers has been extremely powerful for analytical purposes, the low concentration of ions has been a major obstacle for optical spectroscopy. With the help of cooled ion traps and ion storage rings, this problem is finally being resolved, and frequency domain information of ions has just started to emerge in the literature [53,56,57]. Given the vast scope of work, it is needless to say that a comprehensive review of the field of gas phase spectroscopy of biological molecules is practically impossible. In this article, the authors will only concentrate on linear dichroism spectroscopy of gas phase biological molecules. Interested readers are referred to several recent reviews in the literature for a broader perspective [4,24,25,50,51,58,59].

Linear dichroism spectroscopy is typically practiced in the condensed phase to obtain qualitative information on the conformation of fibrous proteins and DNA [60–62]. The sample is aligned in a liquid flow field or in a mechanically stretched film, and the

excitation light source is polarized either parallel or perpendicular to the alignment direction. The difference in absorption or reflection between the two perpendicular polarization directions of the incident light is the LD spectrum of the sample. Adaptation of LD for studies of gas phase biological molecules can establish an ideal bridge between the two distinct fields. Investigations in the condensed phase and gas phase and of solvent/solute complexes will ultimately reveal the intrinsic and extrinsic properties of the relevant biological system. Over the last decade, both technological and theoretical progress has been made [29,37,63,64], and gas phase LD can now be modeled with precision based on quantum or classical mechanics. The direction of the transition dipole derived from LD offers an additional parameter in assisting with spectroscopic assignment. For biological molecules, this additional parameter is crucial because many molecules do not have resolvable resonance lines or have too many congested resonance lines. In these cases, LD often offers the one and only solution.

In this article, we will first review the theoretical treatment of molecular alignment using an external electric field, highlighting the conflicting requirement of alignment and vaporization on the internal temperature of the sample. This problem is resolved by introducing superfluid helium droplets, which can lower the internal temperature of the embedded sample molecule to 0.38 K and moderate the heating temperature due to its high pickup efficiency. A few practical concerns associated with helium droplets will be explained, including the effect of the helium matrix. In the final sections, two types of applications, including electronic and vibrational LD, will be introduced. Most of the results are based on reports from the Miller group and the Kong group [28,29,37,38,63–69].

2. Theoretical background on polarization spectroscopy

While the frequency domain information has proven valuable for determination of secondary structures of gas phase species [1–8], for large molecules with many vibrational modes and small rotational constants, more experimental observables are desired and necessary. Problems plaguing gas phase spectroscopy of biological molecules include spectral line congestion, lifetime broadening due to internal conversion and intersystem crossing, and insensitivity of spectroscopic constants to structural details. Polarization spectroscopy including linear and circular dichroism offers an additional parameter of observation [70–74], thus it is considered an important addition to gas phase spectroscopy. However, in adapting polarization spectroscopy for gas phase studies, CD is challenged by the achievable sample concentration in a gaseous medium, despite several successes reported in the literature [30–34]. For LD fortunately, only sample alignment is required [75,76]. Hence over the last decade, LD has been actively pursued [28,29,37,38,63–69].

The first requirement of LD is sample alignment. In the condensed phase, this is achieved using thin films or flowing solvents to stretch and align the fibrous material [61,77]. In the gas phase, alignment can be achieved using an electric or magnetic field. Here we make a distinction between the terms alignment and orientation; while the former refers to the degree of parallel arrangements, the latter refers to the directionality of vectors. Orientation of polar gas phase molecules in a quadrupole, hexapole, or a uniform electric field, has been developed over the past 40 years [78–84]. A non-uniform field selects a few sub-rotational states to achieve orientation, while a uniform field traps all rotational states of the overall population. The latter is thus achievable only for low temperature

samples such as those from a supersonic expansion [82,84]. This is because the electrostatic interaction between the permanent dipole and the electric field needs to overcome the free molecular rotation, trapping the molecules in pendular states [85,86]. While this technique was applied initially to separate fragments from photodissociation of oriented parent molecules [87,88], its potential for sample alignment was realized by the Kong group in studies of transition dipoles of small molecules [89–97]. Kong's group has since developed the theory to calculate the degree of order in a uniform electric field for small molecules, and the simulation program to model rotationally resolved pendular state spectroscopy [29,63,98]. The full quantum mechanical treatment is crucial for small molecules with large rotational constants at a low temperature, for most biologically related species, on the other hand, classical theory is sufficient. As pointed out by Pei *et al.* [37] when the most populated rotational state is higher than the level with $J = 7$, where J is the total rotational quantum number, the Honl–London factor converges to a constant value for the different rotational branches, and the error introduced in the classical treatment is negligible.

In the classical regime, the thermal energy can be represented by k_BT, and the electrostatic interaction is $\mu_p E\cos\theta$, where k_B is the Boltzmann constant, T is the temperature, μ_p is the permanent dipole, E is the external field, and θ is the angle between the permanent dipole and the electric field. The ultimate degree of alignment is determined by the ratio between the two energies $x \equiv \mu_p E/k_BT$, termed as the trapping field ratio. Under thermal equilibrium, the distribution function $\mathrm{d}N(\theta)/N$ of the permanent dipole is [99]

$$\frac{\mathrm{d}N(\theta)}{N} = \frac{x\, e^{x\cos\theta}\sin\theta\,\mathrm{d}\theta}{e^x - e^{-x}}, \tag{1}$$

where the factor $(e^x - e^{-x})/x$ is the partition function. In the present case of DC field induced orientation/alignment, alignment is a result of orientation, hence in this article, we sometimes refer to the DC field the orientation or alignment field. The degree of alignment represented by the order parameter P_2, can be calculated by:

$$P_2 = \int_0^\pi P_2(\cos\theta)\frac{\mathrm{d}N(\theta)}{N} = \int_0^\pi \frac{(3\cos^2\theta - 1)}{2}\frac{x\, e^{x\cos\theta}\sin\theta\,\mathrm{d}\theta}{(e^x - e^{-x})} = 1 + \frac{3}{x^2} - 3\frac{e^x + e^{-x}}{x(e^x - e^{-x})}. \tag{2}$$

Condensed phase LD experiments measure the difference in absorption under two perpendicular polarization directions of the excitation light source [70]. In gas phase experiments, ratios of the absorption are typically used, and the term polarization ratio ρ is introduced [63]. For a quantum mechanical system, calculation of the polarization ratio requires calculation of the complete excitation spectrum, which is intensive because of the large number of rotational states introduced by the external field in the Hamiltonian matrix. For a classical system, fortunately, the polarization ratio is determined by the direct projections of the transition dipole along the polarization directions of the excitation light source. Figure 1 shows the coordinate system used in this article, where the laboratory frame (XYZ) is defined by the external orientation field, and the molecular frame (xyz) is defined by the two dipoles. The angle between the permanent dipole μ_p and the transition dipole μ_t is α, and the plane of the two dipoles defines the molecular plane xz. In a linear field, there is no confinement in the azimuthal angles ϕ and χ, hence when

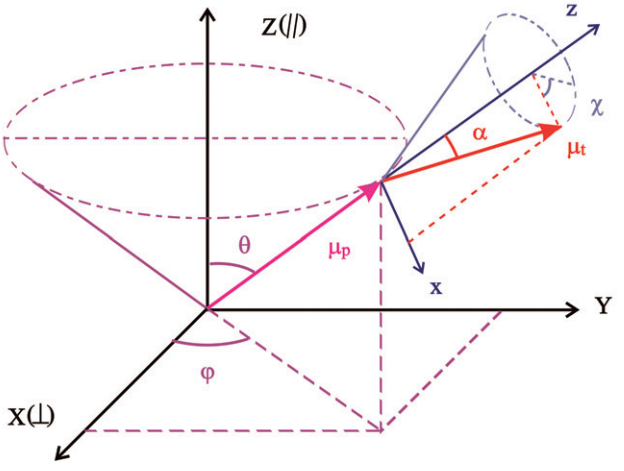

Figure 1. [Colour online] Coordinate system for linear dichroism spectroscopy. The laboratory frame (XYZ) is defined by the alignment electric field, and the molecular frame is defined by the permanent dipole $\mu_p(z)$ and the transition dipole μ_t (xz plane). The angle between the two dipoles is α.

the two dipoles are parallel and the excitation laser is polarized perpendicular to the orientation field, the absorption $A_\perp(0)$ can be obtained from [63]

$$A_\perp(0) = -\frac{1}{x^2} + \frac{e^{2x}+1}{x(e^{2x}-1)}. \tag{3}$$

The polarization ratio for a given angle between the two dipoles can thus be expressed as:

$$\rho/2 = \frac{\cos^2\alpha + A_\perp(0)\cdot(1-3\cos^2\alpha)}{\sin^2\alpha - A_\perp(0)\cdot(1-3\cos^2\alpha)}. \tag{4}$$

Equation (4) is consistent with Equation (25) of Ref. 63, except for the simplicity introduced by $A_\perp(0)$. Figure 2 shows the change of the polarization ratio as a function of the angle between the two dipoles and the trapping field ratio. Typical values of x for small molecules from a supersonic molecular beam are less than one limited by the achievable electric field, but for biological molecules embedded in superfluid helium droplets, x can easily reach several tens or hundreds due to the large permanent dipole and the low rotational temperature. For a given polarization ratio, there is a definitive correlation between the trapping ratio and the angle between the two dipoles. If one of them is known, for example, the permanent dipole and hence the trapping ratio, then the angle between the two dipoles and hence the direction of the transition dipole can be determined. Similarly, if the direction of the transition dipole relative to that of the permanent dipole is known, the permanent dipole can be determined. On the other hand, for a given molecular system with a fixed angle between the two dipoles and a known permanent dipole, the polarization ratio as a function of the applied alignment field traces a line with a unique curvature. Thus experimental measurements of the polarization ratio in different alignment fields can simultaneously determine both the angle between the two dipoles and the size of the permanent dipole [67]. In fact, an advantage of LD in the gas phase over that in the condensed phase is the easy control of the degree of alignment. The mathematical rigor in

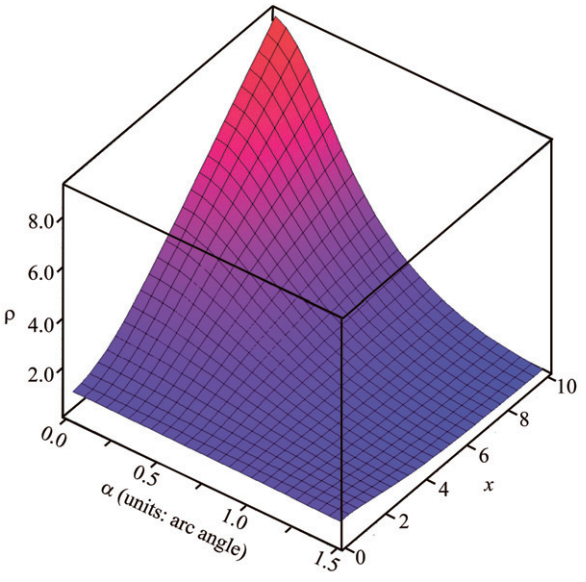

Figure 2. [Colour online] Polarization ratio as a function of the angle between the two dipoles α (units: arc angle) and the trapping field ratio x.

modeling the degree of alignment also offers quantitative information that is rarely available in the condensed phase. In this sense, gas phase LD has the potential of playing an important role in conformational studies, perhaps more so than its counter part in the condensed phase.

Realizing that for electronic transitions of medium and large sized molecules, the direction of the transition dipole is largely unknown, and calculations are often unreliable, particularly for low symmetry species, the Kong group has relied on Equation (4) and Figure 2 to obtain directions of transition dipoles [63,97]. The premise of this application is the knowledge of the size and direction of the permanent dipole of the ground state molecule. Although experimental values of the permanent dipole for non-volatile species typically have an uncertainty on the order of 5–10% [100], theoretical values obtained from high level calculations are largely reliable within 2–5%, as long as polarization basis functions are included [37]. From theoretical permanent dipoles and thereby values of trapping field ratios and experimental polarization ratios, directions of transition dipoles for a number of gas phase molecules have been obtained [89–97].

For biological molecules, however, conformational flexibility typically results in coexistence of several conformers, and each conformer has its unique permanent dipole moment [29,101,102]. The second type of application of Equation (4) thus relies on the knowledge of the electronic transition dipole and derives the permanent dipole for conformational assignment. A correlation between the molecular conformation and its permanent dipole has to be established a priori, largely based on high level theoretical calculations. In addition, the direction of the electronic transition dipole is predetermined by the chromophore in the molecule. The experimental polarization ratio is then used to match the conformation with the observed permanent dipole, as illustrated in the case of tryptamine (Section 5.1) [29].

Similar to the second type of application, Miller's group has developed the vibrational transition moment angle (VTMA) method as an additional observation parameter for vibrational assignment of biological molecules [28]. The direction of the transition dipole of an observed IR band is measured, and comparisons of the angle between the transition and permanent dipoles from experiment and from high level calculations can lead to a definitive assignment of a vibrational mode for a particular tautomer. This method is powerful in two situations, one is when different vibrational modes of the same tautomer have similar frequencies, and the other is when different tautomers have similar vibrational frequencies. Although in the latter case, IR-UV hole-burning experiments can separate transitions that belong to the same tautomer, structural assignment of each tautomer is still difficult if possible at all. In applying this method for vibrational spectroscopy of nucleic acid bases, the Miller group has also noticed that in some instances, the direction of the transition dipole is sensitive to details of the molecular geometry, and discrepancies in VTMA between theory and experiment can occur [65,67]. In these cases, Miller and company measured variations of the polarization ratio as a function of the alignment field, and comparisons of the curvature of the polarization ratio offered more supportive information for vibrational assignment. Most biological molecules have many vibrational modes congested in the IR, thus the impact of VTMA could be tremendous.

It is important to note that the above calculation assumes polar molecules with a permanent dipole μ_p, and similar calculations can be performed for non-polar molecules with a polarizability anisotropy $\Delta\alpha$ in an external field [103–106]. In this case, the trapping field ratio becomes $x \equiv \Delta\alpha\, E^2/k_B T$. For most small molecules, however, this polarizability factor is much weaker than that of the corresponding permanent dipole. Even in a field of 100 kV/cm with a polarizability volume of 200 Å^3 (phthalocyanine for example), the induced dipole is only 0.01 debye, far smaller than most permanent dipoles and smaller than the uncertainty of the permanent dipole for many non-volatile species [100]. The effect of the polarizability anisotropy is therefore only considered in strong laser fields (10^4 kV/cm) with laser intensities over 10^{12} W/cm^2.

3. A few practical concerns

DC Field induced orientation and alignment rely on the low rotational temperature of the sample. Unfortunately, for non-volatile biological molecules, this is difficult to achieve due to the extreme measures that have to be taken for vaporization. Miller's group first introduced continuous superfluid helium droplets for cooling of the non-volatile species [28,107]. Kong's group has adopted a pulsed beam for the same effect [29]. Although fluctuations of the pulsed nozzle used in the Kong group are problematic for frequency domain spectroscopy, with sufficient averaging and adequate measures, some successes have been achieved [29,37,38]. A superior advantage of embedding non-volatile species in superfluid helium droplets is the high pickup efficiency of the droplet beam: vapor pressures in the range of 10^{-6}–10^{-5} torr are proven sufficient for optical spectroscopy of doped helium droplets [108–110]. The required heating temperature of a droplet experiment is thus considerably lower than that for a typical gas phase experiment, and many thermally labile molecules that are not suitable for gas phase experiments, such as guanine, can be studied in a droplet experiment [67].

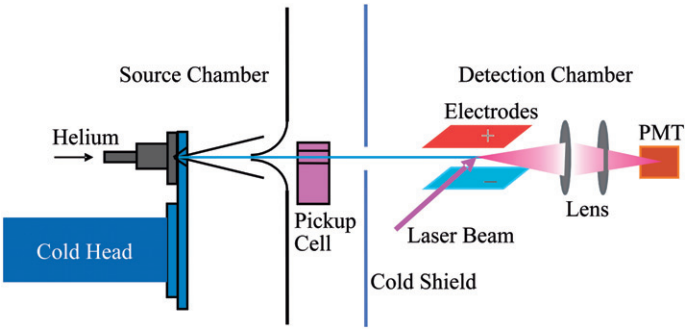

Figure 3. [Colour online] Experimental apparatus for linear dichroism spectroscopy of gas phase biological molecules using superfluid helium droplets for cooling and a DC electric field for alignment. High pressure helium is precooled and expands into high vacuum through a pulsed nozzle, forming superfluid helium droplets. The sample is introduced from the pickup cell, and fluorescence of the embedded sample is detected by a photomultiplier tube (PMT).

Figure 3 shows the experimental setup from the Kong group for polarization spectroscopy of biological molecules embedded in superfluid helium droplets. It is based on the report of Ref. 111 with several modifications. The setup consists of two differentially pumped chambers. The source chamber is pumped by a diffusion pump of 3500 l/s, and the detection chamber is pumped by a turbo molecular pump of 800 l/s. The static vacuum in both chambers is about 2×10^{-7} torr. Superfluid helium droplets are generated by expansion of helium (purity: 99.5%) at a stagnation pressure of 15 atm through a 0.3 mm pulsed nozzle. The nozzle is cooled to 18 K by a two-stage closed cycle cryocooler system (RDK408S, Sumitomo Heavy Industries, Ltd). The temperature of the pulsed nozzle is measured by a silicon diode sensor (Lakeshore Cryogenics) and controlled by a temperature controller (Cryo-Con 32B). The droplet beam is collimated by a 2.0 mm skimmer before entering into the orientation/detection chamber. The pickup cell is located at the exit of the skimmer with two 4 mm holes for the passing droplet beam. About 20 cm downstream from the pickup cell, the droplet beam is intercepted by an excitation laser.

During the experiment, we have discovered that a low background pressure is essential because of the high pickup efficiency of the droplet beam: residual gases can dominate the pickup process when the vapor pressure of the sample is low, or they can complex with the sample and generate interferences. In an extreme case of high background pressure (10^{-5} torr), the droplet beam can even be destroyed completely during pickup and evaporative cooling. Thus the high pickup efficiency of the droplet beam is also a mixed blessing: an oil and water free vacuum almost to the level of ultrahigh vacuum is needed for a successful experiment.

Several different detection methods have been reported in the literature for optical spectroscopy of doped helium droplet beams [108–110]. The most commonly used is depletion of the He_2 mass from a quadrupole mass spectrometer [108,111]. For polarization studies, Miller's group used a bolometer for absorption in the infrared [110], and Kong's group used laser induced fluorescence (LIF) for absorption in the UV/VIS [29,37,38]. The pulsed operation mode of the droplet apparatus from the Kong

group poses a technical challenge for quadrupole mass spectrometers, and a pulsed electron gun is required for ionization.

Effects of the superfluid helium matrix on the spectroscopy of the embedded species have been discussed in the literature [29]. In the IR region, there is minimal disturbance to the solvation environment upon vibrational excitation, hence no frequency shifts in the vibrational bands have been reported [110]. On the other hand, many molecules studied in the IR demonstrate no rotational resolution, and homogeneous broadening of the droplet environment has been suspected. Electronic transitions change the size and shape of the electronic cloud of the embedded molecule. Adjustment of the first layer helium atoms that are not in the superfluidic regime upon electronic excitation can activate the relevant phonon wings. Thus in electronic spectroscopy of doped helium droplets, phonon wings are ubiquitous and can sometimes mask the zero phonon line [108–110]. Nevertheless, all phonon wings associated with a zero phonon line originate from the same electronic excitation, and the same polarization dependence has been observed [29,37]. Consequently, although coupling with the droplet environment does exist for electronic transitions, it does not affect the direction of the electronic transition dipole, and Equation (4) is equally applicable for both phonon wings and zero phonon lines.

Miller's group has also reported that the permanent dipole of an embedded species can be slightly smaller than that of the gas phase species [107]. The net reduction is on the order of 2%, and for most molecules, this small difference is below the uncertainty in the permanent dipole and the uncertainty of the experimental measurement of the polarization ratio [29,37].

4. Vibrational transition moment angle

Miller's group proposed the idea of vibrational transition moment angle for vibrational assignment of biological molecules and demonstrated its application using nucleic acid bases [28,65–69]. For effective cooling and thereby sample orientation and alignment, they further introduced superfluid helium droplets to embed the non-volatile biological molecules. Unlike electronic transitions where the direction of the transition dipole from theoretical calculations is typically problematic, directions of vibrational transition dipoles for the ground electronic state are insensitive to the basis set and calculation method, as shown in Figure 4 [28]. The idea of VTMA relies on the fact that for a given angle α between the permanent dipole and the vibrational transition dipole, and for a given size of the permanent dipole, the polarization ratio ρ is only determined by the external field via the trapping ratio x (Figure 2). Matching of the theoretical and experimental VTMA uniquely defines the vibrational mode and the molecular structure. This method expands vibrational spectroscopy into a new dimension, and for biological molecules which typically have many congested vibrational modes and many coexisting structural isomers, it is particularly powerful. In addition, according to the Miller group [28,65–69], frequency information from calculations can be misleading, and in some cases, vibrational frequencies are insensitive to the molecular geometry. Similarly, in microwave spectroscopy, the rotational constants from calculation may be similar for different molecular structures, and comparisons with experimental values are difficult for a definitive assignment [112–114]. In these cases, VTMA becomes the last resource.

Reports of VTMA measurements center around four vibrational modes, including NH_2 (symmetric stretch, ss), NH_2 (asymmetric stretch, as), free OH stretch, and free NH stretch [28,65–69]. In all cases, the polarization dependence of the IR transition on the external electric field offers conclusive evidence for vibrational and tautomeric assignment. For example, in the case of adenine [69], three closely spaced features were observed near the symmetric stretching mode of NH_2, but only one of them demonstrated the predicted dependence on an external field. Consequently, the two other bands were attributed to contributions from higher energy tautomers or clusters. In addition, although a few other studies offered tantalizing and conflicting evidence for the non-planarity of adenine, the analysis from Choi *et al.* was one of the most convincing [69]. Both the sensitivity of VTMA to the detailed orientation of the relevant chemical bond and simultaneous matching of the VTMA for all three observed vibrational bands confirmed the non-planarity of the amine group. In another example, three major tautomers of cytosine were observed both in the gas phase and from superfluid helium droplets [66,114–116]. The gas phase study in the microwave region relied on the obtained rotational constants and extensive calculations for tautomeric assignment, some of which were extrapolated to the limit of a complete basis set [114–116]. However, the root-mean-square deviations of the rotational constants between theory and experiment were dependent on the basis set and

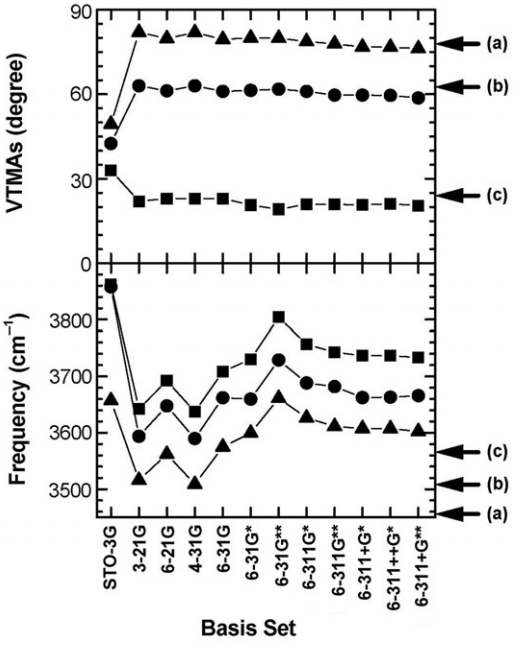

Figure 4. Basis set dependence of the vibrational frequencies (lower panel) and transition dipole angles (VTMA, upper panel) for three modes of adenine [28]. The horizontal arrows indicate the experimental frequencies and VTMAs. While the frequencies fluctuate with the basis set and differ from the experimental value, the VTMA converges to the experimental value and remains unchanged with the increase of the basis set. Triangles and (a): NH_2 symmetric stretch; circles and (b): N-H stretch; squares and (c): NH_2 asymmetric stretch.

calculation method, and different methods converged to different structures. In contrast, the droplet study by Choi, Dong, and Miller was based on VTMA [66], and their conclusion was independent of the calculation method and the basis set. The discrepancy between the conclusions of the gas phase and those of the droplet was therefore attributed to the insensitivity of rotational constants to the detailed molecular structure within the accuracy of theoretical calculations and experimental results. This example manifests the power of additional parameters for spectroscopic assignments, and the caution one should exert in referencing computational values.

The report on guanine is interesting in several aspects [67]. First, theoretical vibrational frequencies for the OH group in the enol forms were higher than those of the NH_2 asymmetric stretching by over 60 cm^{-1}, but VTMA analyses revealed that these two bands were clustered together. The OH band was considerably lower in frequency than predicted, while the asymmetric NH_2 band was higher. No scaling factor could thus be derived, and tautomeric and vibrational mode assignment became ambiguous if only frequency information was used. Second, for the N1H stretching mode of two tautomers, a larger than usual error in the VTMA was observed. The authors attributed this discrepancy to the sensitivity of VTMA on the molecular geometry, since the dihedral angle of the related bonds was sensitive to the calculation basis set. To further confirm the assignment in this case, the authors traced the change of the transition intensity ratio as a function of the external field, as shown in Figure 5. This ratio was defined as the signal intensity ratio with and without the alignment field when the polarization direction of the laser is parallel to

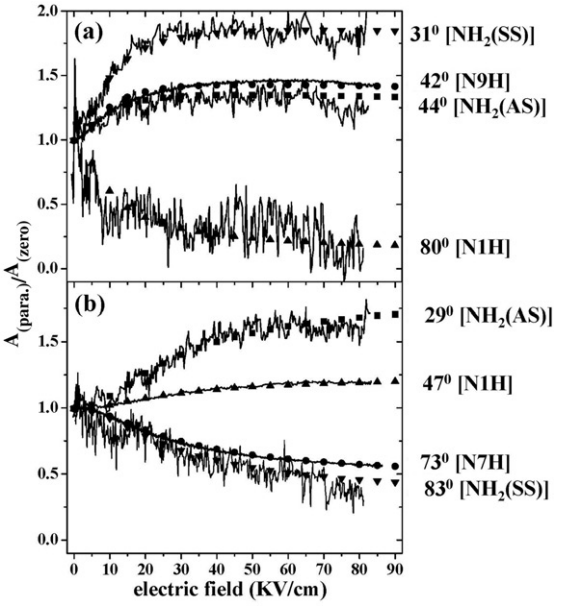

Figure 5. Transition intensity ratios as a function of the electric field for four vibrational bands of the (a) G9K and (b) G7K tautomers of guanine [67]. The ratios refer to the signal strength with and without the electric field and with the polarization direction of the laser field parallel to the alignment. The solid lines show the experimental results, while the symbols correspond to the calculations based on the *ab initio* dipole moment of 6.26 (G9K) and 1.88 D (G7K).

the alignment field. By relying on the curvature of this ratio similar to the procedure explained in Section 2, the authors simultaneously determined the direction of the transition dipole and the size of the permanent dipole, thereby confirming the vibrational and tautomeric assignment. Third, from the vibrational assignment and theoretical transition moment amplitudes, the authors were able to derive a population distribution for the four observed tautomers. Not too surprisingly, the population distribution from VTMA disagreed with that obtained from gas phase studies [42,117–120]. Different from the case of cytosine, however, this discrepancy was not related to the ambiguity in vibrational assignment, since different vibrational spectra were obtained in the gas phase and from doped droplets. Rather, different tautomers were believed to exist in the different sample sources.

In addition to monomers, Miller's group has also used the same approach to investigate homogeneous clusters and water complexes [65,121]. For systems containing weak inter- and intra-molecular forces, calculations are often challenging, even with unlimited computational resources. VTMA is therefore indispensable for spectroscopic assignment. For example, the ordering of the vibrational frequencies for the four observed uracil·water complexes was wrong from the *ab initio* calculation at the MP2/6-311++G*(d,p) level [65]. Choi and Miller thus proposed that perhaps a method of geometry optimization based on experimental values of VTMA could be developed.

5. Electronic linear dichroism for conformation studies

5.1. *Tryptamine*

Electronic linear dichroism spectroscopy in the gas phase relies on the known transition dipole moment and determines the permanent dipole from experimental measurements of the polarization ratio. Conformation assignment is achieved from the established relation between the permanent dipole and the molecular conformation based on *ab initio* or density functional theory (DFT) calculations. Pei *et al*. reported the first conformational study of biological molecules using LD in the gas phase [29]. Tryptamine contains an indole chromophore and a polar side chain. The direction of the transition dipole of indole is known, but the size and direction of the permanent dipole are determined by the conformation of the ethylamine size chain [102]. Conformational assignment of the observed electronic transitions of tryptamine is therefore an ideal application for LD.

Figure 6 shows the laser induced fluorescence spectra of tryptamine embedded in superfluid helium droplets. Among the three observed major conformers A, D, and E, the structures for conformers A and E have been determined using high resolution spectroscopy [112,122,123], while the two candidates for structure D are difficult to distinguish due to similarities in rotational constants from theoretical calculations [112,113]. In fact, two independent studies based on rotationally resolved spectroscopy arrived at two different conclusions [112,113]. In the Anti(py) structure, the angle between the two dipoles is close to the magic angle (180°–$127^\circ = 53^\circ$), while in the Anti(ph) structure, this angle is close to 90°. In Figure 7, the variation of the polarization ratio as a function of the external field is plotted for each conformation and for each possible direction of the transition dipole [29]. It is interesting to note that high resolution spectroscopy can determine the absolute projection of the transition dipole in the molecular frame, but the sign of each projection is still unknown, and all four possibilities

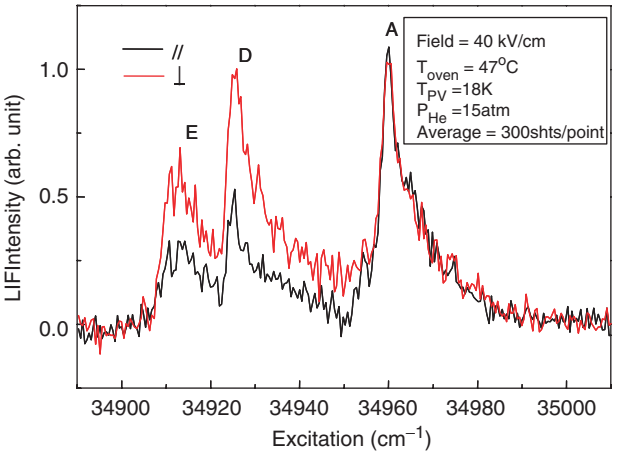

Figure 6. [Colour online] LIF spectra of embedded tryptamine in an orientation field of 40 kV/cm. The spectra were recorded when the polarization direction of the excitation laser was parallel (//) and perpendicular (⊥) to the alignment electric field.

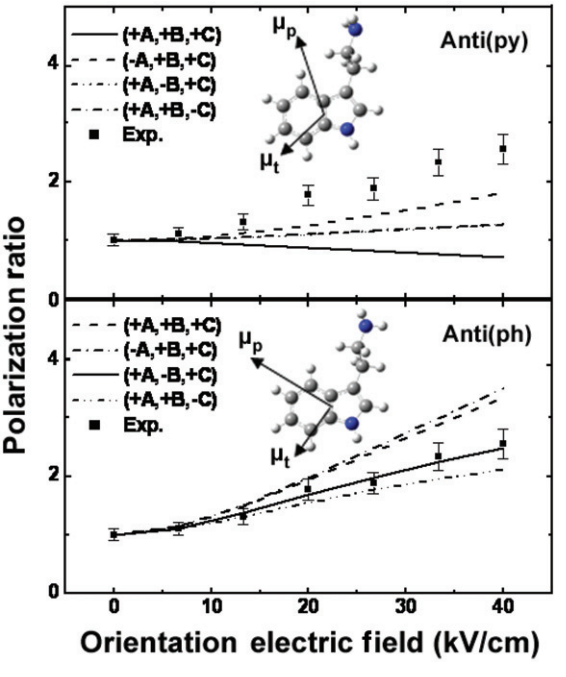

Figure 7. [Colour online] Dependence of the polarization ratio on the external electric field for conformer D of tryptamine. Theoretical values assuming different projections of the transition dipole are overlayed with the experimental data. From this comparison, the Anti(py) structure is eliminated, while only the Anti(ph) structure agrees with the experimental result.

in Figure 7 are necessary for a definitive assignment. The observed strong polarization preference confirms the Anti(ph) structure for conformer D. This work exemplifies the power of polarization spectroscopy, since given the similarities of the two candidate structures for conformer D, this method is the only approach currently available for this type of structural assignment.

5.2. *The search of a chromophore*

In using LIF for conformational studies of biological molecules, a bright chromophore is needed. Although this requirement is easily met in the gas phase, superfluid helium droplets pose an additional challenge. This is because only a few molecules are known to have sufficient fluorescence in a droplet environment, while some species have decreased fluorescence yields [108–110]. In addition to indole, the Kong group has explored a variety of chromophores [29,38]. In particular, tetracene and phthalocyanine (Pc) are convenient in excitation wavelength and have exhibited high fluorescence yields in a droplet environment, therefore they are more favorable than indole. However, a detailed study of the Pc chromophore has revealed a potential complication. Figure 8 shows the polarization spectroscopy of chloroaluminum phthalocyanine (AlPcCl) and chlorogallium phthalocyanine (GaPcCl). In these compounds, the ionic component AlCl or GaCl is covalently attached to the Pc chromophore, as shown in the inset of Figure 8, and its function is to mimic a polar biological molecule by offering a permanent dipole. The C_{4v} symmetry of the system dictates that the permanent dipole should be perpendicular to the Pc plane, while the transition dipole of the chromophore should be within the Pc plane [37]. The dotted lines in Figure 8 represent the calculation results by assuming such a perpendicular relationship between the two dipoles, and the disagreement between this model and the experimental data is obvious. By assuming an angle of $\sim 70^\circ$ between the two dipoles as shown by the solid lines, calculation and experiment agree reasonably well.

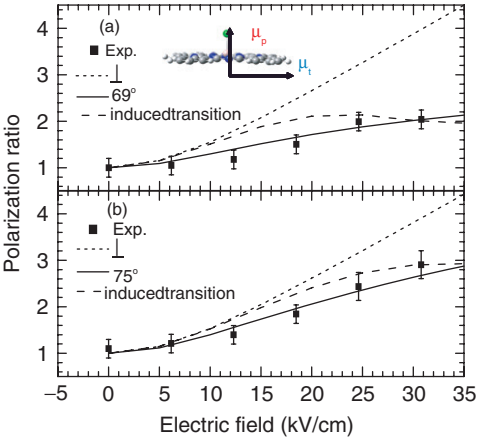

Figure 8. [Colour online] Polarization ratios of AlPcCl (a) and GaPcCl (b) under different external fields. The dotted lines are calculation results assuming a perpendicular relation between the two dipoles, the dashed lines are results assuming an additional induced dipole out of the Pc plane, and the solid lines are results assuming an intrinsic out of plane component in the Pc chromophore.

The dashed lines are results of assuming an induced dipole by the orientation field, and the induced out of plane transition dipole is linearly dependent on the external field. The comparison in Figure 8 further suggests that the transition dipole in MPcCl compounds is not related to the external field; rather, it is intrinsically titled out of the Pc plane.

According to Figure 2, there are two parameters that determine the theoretical polarization ratio, the size of the permanent dipole and the angle between the permanent and the transition dipole. To assess the reliability of the permanent dipole obtained from our DFT calculation, Table 1 shows the permanent dipoles of aluminum porphyrin chloride and gallium porphyrin chloride obtained using a variety of basis sets, from 3–21G to 6–311G, with and without polarization orbitals. The structure of porphyrin is similar to that of phthalocyanine, but it contains much fewer atoms and the calculation is thus possible with a limited computational resource. The values in Table 1 suggest that as long as polarization orbitals are included, the variation in size for the permanent dipole is within 2% of the average value, although without any polarization orbital, the size of the permanent dipole is consistently larger by about 20%. For the two Pc compounds, we have obtained results using two different basis sets and two different methods limited by our computational resources. The results using the 6–31G** basis set are essentially the same as those obtained using the 3–21G* basis set, in agreement with the conclusion of Table 1. The MP2 results after full geometry optimization are also similar, although for GaPcCl, the difference is slightly larger by about 5%. Nevertheless, these differences in theoretical values are insignificant in comparison with the uncertainty of the experimental value (20%) and the typical uncertainty in experimental measurements of permanent dipoles [100]. On the other hand, to reproduce the experimental ratio for AlPcCl, a permanent dipole of 2 debye is needed. This value is too small to be credible based on the consideration of the charge distribution and the bond length of Al-Cl. The experimental value for the bond length of Al-Cl is between 2.06 and 2.13 Å based on the Computational Chemistry Comparison and Benchmark DataBase published by NIST (http://srdata.nist.gov/cccbdb/). If we assume a complete electron transfer to the chlorine atom, the permanent dipole of the Al-Cl bond would be on the order of 10 debye, similar to that of NaCl. The much smaller value of 4 debye for AlPcCl from our calculation is a result of a much smaller charge on the chlorine atom, about 0.4 unit for AlPcCl and 0.3 for GaPcCl

Table 1. Permanent dipole moments of metal porphyrin chloride (MPhCl, M = Al, Ga) calculated using the B3LYP functional at different levels [37].

Basic set	AlPhCl	Deviation (%)	GaPhCl	Deviation (%)
3–21G*	4.0165	1.4	3.9233	−0.2
3–21G**	4.0063	1.2	3.9134	−0.4
6–31G*	3.8912	−1.7	3.9122	−0.5
6–31G**	3.8920	−1.7	3.9128	−0.5
6–311G*	3.9830	0.6	3.9813	1.3
6–311G**	3.9721	0.3	3.9404	0.2
Average	3.9602		3.9306	
6–31G	4.8895		4.7107	
3–21G	5.0556		4.9204	
6–311G	4.7270		4.5437	

from our calculation. In order to decrease the permanent dipole to 2 debye in AlPcCl, the bond length would have to be considerably shortened, and the charge on the chlorine atom would have to be further reduced. Based on these considerations, the size of the permanent dipole for the two metal phthalocyanine chlorides is unlikely the major reason for the discrepancy between theory and experiment. Furthermore, although these permanent dipoles are only gas phase values, the corresponding values for the embedded species should be similar within a few percent, resulting in negligible effects on the polarization ratio [107].

From the above discussion, the origin of the discrepancy between experiment and theory can only be attributed to the angle between the transition dipole and the permanent dipole, i.e., the transition dipole has an additional component out of the Pc plane. Two possible reasons for the existence of such a component have been suggested [37]: one is the $n\pi^*$ transition involving the chloride, and the other involves the non-bonding orbitals in the phthalocyanine chromophore. In order to further distinguish these two origins, the Kong group has investigated the polarization spectroscopy of AlPcOH [38]. The chloride is replaced by a hydroxide therefore both the orientation and energy of the non-bonding orbitals are changed. Although additional complication is introduced due to the low symmetry of AlPcOH, quantitative analysis and comparisons with high level calculations have concluded on a ~10% out of plane contribution in the transition dipole. This result excludes the contribution of the chloride to the out of plane component in the transition dipole. Thus the most likely origin of this component is the non-bonding orbitals on the bridge nitrogen atoms in the Pc chromophore, perhaps induced by the charged out of plane central metal atom.

In addition to considerations of the fluorescence yield in a superfluid helium environment and the excitation wavelength, another important criterion for the selection of chromophores in this type of LD experiment is the synthetic convenience of coupling the chromophore with the biological molecule. In this sense, both tetracene and phthalocyanine pose some challenges, since they require more than two or three synthetic steps to couple with any biological molecule. A better candidate just from this perspective is the fluorene chromophore typically attached to commercial amino acids as a protective group in the form of 9-fluorenylmethyl carbamate (FMOC). Gas phase LIF studies of fluorene have been reported [124], and LIF experiments of FMOC related compounds in superfluid helium droplets are being actively pursued in the Kong group.

A superior advantage of conformational identification using electronic LD is the unlimited size of the investigation target. Molecules large and small, with or without line spectra, can be interrogated using this method. No spectroscopic assignment of the observed transition is necessary, and for a known chromophore, only modeling of the permanent dipole of the ground electronic state is required, while no calculation of the excited state is necessary. The additional savings in human power and computational power become increasingly important as the investigation target increases to larger and larger biological systems.

6. Outlook

Polarization spectroscopy both in the UV/VIS and IR is complementary to other types of spectroscopy. In particular, the direction of a vibrational transition dipole is proven more

reliable than energies and frequencies from theoretical calculations. Thus VTMA is promising in expanding the scope of applications of vibrational spectroscopy. For electronic LD, reliable permanent dipoles of the ground state can be obtained from theory, thus conformational analysis based on the permanent dipole is definitive and straightforward, and it does not rely on detailed rovibrational assignment. The DC orientation method is easily achievable in many laboratories, although the superfluid helium droplet source is challenging. On the other hand, to make significant progress in gas phase spectroscopic investigations of biological molecules, this investment is worthwhile. With the success in vaporization methods either through laser desorption or direct heating, the technical frontier of optical spectroscopy of biological molecules shifts to development of new experimental observables, and LD is just one example.

In the solution phase, biological studies have benefited from polarization information extensively. Gas phase studies can directly assist with solution phase studies in offering the corresponding information of the isolated molecule. The move of adding water molecules to isolated species can further elucidate the effect of the solvent in modifying the chemical and physical properties of the solute. The unlimited size range of gas phase LD is tremendously beneficial as the field progresses to larger and larger molecular systems. The mathematical rigor and the ease in controlling the degree of alignment both make gas phase LD even more attractive than its counterpart in the condensed phase.

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