Solvatochromism and the solvation structure of benzophenone

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Many complex molecular phenomena, including macromolecular association, protein folding, and chemical reactivity, are determined by the nuances of their electrostatic landscapes. The measurement of such electrostatic effects is nonetheless difficult, and is typically accomplished by exploiting a spectroscopic probe within the system of interest, such as through the vibrational Stark effect. Raman spectroscopy and solvatochromism afford an alternative to this method, circumventing the limitations of infrared spectroscopy, providing a lower detection limit, and permitting measurement in a native chemical environment. To explore this possibility, the solvatochromism of the C=O and aromatic C–H stretching modes of benzophenone are investigated using Raman spectroscopy. In conjunction with density functional theory calculations, these observations are sufficient to determine the probe electrostatic environment as well as contributions from halogen and hydrogen bonding. Further analysis using a detailed Kubo–Anderson lineshape model permits the detailed assignment of distinct hydrogen bonding configurations for water in the benzophenone solvation shell. These observations reinforce the use of benzophenone as an effective electrostatic probe for complex chemical systems.

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I. INTRODUCTION

Solvatochromism has become well-established as a technique to measure the local electrostatic environment within chemical systems. When encapsulated in a continuum dielectric, a probe molecule will experience an effective electric field $\vec{E}_{\text{env}}$ due to the surrounding medium such as solvent and other co-embedded molecular entities including lipids, proteins, and ions. This field perturbs the active electronic or vibrational bands of the probe, inducing a change in energy $\Delta E_{\text{solv}} = hc\Delta\nu = -\Delta\vec{\mu} \cdot \vec{E}_{\text{env}}$, where $\Delta\vec{\mu}$ is the shift in dipole moment associated with the spectroscopic transition for this mode. The corresponding frequency shift $\Delta\nu$ of a given band is termed the solvatochromic shift. Thus, given a spectroscopic measurement of the band frequency, an estimate of $\Delta\vec{\mu}$, and some knowledge of the probe orientation, the electrostatics may be precisely defined. Due to the inverse square nature of electrostatic interactions, this effect is sensitive to local environmental changes. Such measurements are obviously of high utility in biomolecular systems, where ligand binding and macromolecular association are accompanied by dramatic alterations in the molecular environment.

The most common technique for measurement of $\Delta\vec{\mu}$ is Stark spectroscopy, in which the system is placed in an external, static, spatially-homogeneous electric field.¹ While the electronic Stark effect has traditionally served as the method of choice for a biomolecular systems, the vibrational Stark effect has recently enjoyed some popularity.² As the name implies, the vibrational Stark effect exploits the perturbation of a vibrationally active mode in the probe molecule, which is typically interrogated using infrared absorption spectroscopy. The absorption spectrum is obtained for a system in the pres-

ence and in the absence of an applied field, and the difference spectrum utilized to derive $|\Delta\mu|/h$, termed the vibrational Stark tuning rate. While first employed to probe biomolecular electrostatics in conjunction with diatomic ligands,³ this method was later expanded to include nitrile-based small molecule probes and unnatural amino acids^{2,4-9} as well as ionic liquids.¹⁰ These studies demonstrate that electrochromic probes afford a method to monitor processes such as protein folding and the conformational changes associated with ligand binding and catalysis without the heavy preparative overhead of techniques such as NMR spectroscopy. Furthermore, a detailed picture of the probe environment may be resolved by utilizing quantum mechanical/molecular mechanical (QM/MM) density functional theory (DFT) calculations¹¹ in tandem with experiment, as recently demonstrated for a thiocyanate vibrational marker.¹²⁻¹⁶

While the aforementioned nitrile probes are effective and well characterized,^{17,18} they comprise only a small region of chemical parameter space. In fact, other nitrile and nitrile-free probes including alcohols, azides, fluorides, nitrosyl, and carbonyl compounds have been investigated both experimentally^{2,4,19,20} and theoretically.²¹⁻²⁵ Furthermore, the aforementioned studies have all utilized the Stark effect, with a few notable exceptions^{9,10} in which the nitrile solvatochromic shift was calibrated against a standard series of solvents. The latter approach is ideal as it drastically simplifies the measurement technique and permits observations over a broad range of physiologically relevant conditions. The utility of solvatochromic probes is likewise enhanced by using other spectroscopic techniques such as UV Raman spectroscopy, where the detection limit of a particular nitrile probe has been shown to lie 100-fold lower in concentration than during the corresponding infrared measurements. The environmental sensitivity of this probe was likewise retained, as

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practically demonstrated through the detailed characterization of liposome association of a small peptide.²⁶ In many cases, the use of Raman methods is preferable to infrared spectroscopy due to greater optical penetration of the sample, coupled with the weak contribution of water to this process.

Despite the advantages afforded by Raman spectroscopy, the absence of an external field can complicate the detailed interpretation of these shifts. In a general solvatochromic process, the environmentally induced vibrational shift $\Delta\nu$ may be expanded as a Taylor series

$$hc\Delta\nu = -\Delta\vec{\mu} \cdot \vec{E}_{\text{env}} - \frac{1}{2}\vec{E}_{\text{env}} \cdot \hat{\alpha} \cdot \vec{E}_{\text{env}} + \dots, \quad (1)$$

where $\hat{\alpha}$ is the polarization tensor for the system. In the case of a Stark effect measurement, the environmental field decomposes into contributions arising from the solvent $\vec{E}_{\text{solv}}$ and the applied electric field $\vec{E}_{\text{appl}}$ as $\vec{E}_{\text{env}} = \vec{E}_{\text{appl}} + \vec{E}_{\text{solv}}$. This applied field is typically small with respect to the local solvent field. Nonetheless, in conjunction with matrix isolation, the external field induces spatial homogeneity into the system. Thus, the first order term in Eq. (1) is generally sufficient to describe the system in terms of the solvent field alone during a Stark effect observation. In the case of a Raman measurement, this external field is absent and the electric field arising from the solvent is strongly anisotropic. Furthermore, unlike absorptive infrared spectroscopy, Raman measurements induce additional environmental polarization and hence higher order contributions to $\Delta\nu$ may be relevant. DFT and QM/MM DFT studies at the B3LYP/6-311++G(d,p) level using a series of nitrile, halide, and carbonyl based probes indicate that this linear relationship is only retained for the halide and carbonyl probes in the absence of an external field.²⁴ Accordingly, the popular nitrile-based probes may have poor applicability to Raman observations of solvatochromism.

An ideal alternative is afforded by using the carbonyl stretching mode of benzophenone as a vibrational marker. While the UV solvatochromism of benzophenone and its derivatives has been thoroughly characterized,²⁷⁻²⁹ the corresponding vibrational shifts have only been systematically explored in the infrared.³⁰ Benzophenone has nonetheless proven effective as a vibrational probe for the solvatochromic and charge transfer properties of photovoltaic materials.²⁰ In fact, the singlet and triplet Raman active modes of benzophenone are already employed as an efficacious tool to monitor reaction dynamics during time-resolved spectroscopy.^{8,31-33} The robustness of Raman in terms of low detection limits, strong sample penetration, and the silence of water within the visible spectrum, coupled with the versatility of benzophenone, makes this tool attractive as a probe of biomolecular dynamics.

In order to establish benzophenone as a Raman-active solvatochromic probe, a systematic accounting of its solvation properties must be performed. Herein, the $\nu(\text{C}=\text{O})$ vibrational band, corresponding to the carbonyl stretching mode, is calibrated against a series of solvents of disparate dielectric strengths. In conjunction with DFT calculations, it is demonstrated that contributions from either hydrogen or halogen bonding to the carbonyl may be dissected from solvation effects, and a precise assignment made of the encapsulating

dielectric environment. Furthermore, by titrating water into aprotic solutions of benzophenone, the solvation structure of a benzophenone molecule may be assigned from lineshape analysis of spectroscopic measurements. These observations collectively demonstrate the utility of benzophenone as a vibrational probe of electrostatics as applicable to a diverse spectrum of chemical systems.

II. EXPERIMENTAL AND COMPUTATIONAL METHODS

A. Raman spectroscopy

Standardized 100 mM benzophenone solutions were prepared in commercial HPLC or spectrophotometric grade solvents, excepting hexane, which was prepared at the solubility limit of 15 mM. This concentration was chosen to afford a maximum of detectability while avoiding solute self-association, as determined by ensuring that the $\nu(\text{C}=\text{O})$ band did not change with increasing solute concentration (see the supplementary material³⁴). Raman spectra were obtained from excitation using the 441.6 nm line of a CW HeCd⁺ laser (Kimmon Electric) and stray spectral lines removed using a 441.6 nm laser line bandpass filter. The orientation of the linearly polarized laser light was determined and fixed using a half wave plate. The incident beam was directed onto the benzophenone sample solution, contained in a 10.0 mm $\times$ 4.0 mm quartz cell for solvatochromic measurements or a 10.0 mm $\times$ 10.0 mm cell for titration measurements. Raman scattered light was collected in a 90° geometry relative to the exciting radiation and imaged onto the monochromator using $f = 10.0$ cm and $f = 5.0$ cm quartz lenses. The polarization was analyzed using a polarizing beamsplitter, and passed through a polarization scrambler and 442 nm longpass filter to eliminate stray light from the laser line. Spectra were collected using a $f/9.7$ single grating monochromator (Acton SP2750, Princeton Instruments) at 15 μm slit width using 1200 g mm⁻¹ and 1800 g mm⁻¹ gratings and imaged using an LN₂ cooled 1340 $\times$ 400 pixel back-illuminated CCD camera with UV grade coatings (PyLoN 400BR eXcelon, Princeton Instruments). Laser power at the sample was measured to be ~ 30 mW in all cases and spectral data was acquired in 120 s exposures. Measurements were taken at 20 °C unless otherwise specified. Reference calibrations were performed with respect to a Hg vapor lamp standard with an absolute calibration accuracy of 0.004 nm RMS, spatial resolution of ± 0.08 nm, and absolute spectral calibration in the region of interest of ± 1.0 cm⁻¹.

B. Density functional theory calculations

Geometries of benzophenone and benzophenone in complex with explicit solvent were optimized using DFT. The cc-pVTZ³⁵ basis set was adopted for all calculations, unless otherwise specified, and employed in conjunction with the B3LYP³⁶⁻³⁸ density functional as implemented in GAUSSIAN 09.³⁹ Optimizations were performed in vacuum and in implicit solvents using the Polarizable Continuum Model (PCM) to treat the dielectric background.⁴⁰ Vibrational analyses were performed at an identical level of theory.

C. Spectral analysis

The Kubo–Anderson^{41–43} model has been successfully utilized to describe hydrogen bonding dynamics and the local molecular environment associated with a given spectral feature in terms of the Raman lineshape.^{44–46} While similar data may be derived from an empirical analysis,⁴⁷ this formalism affords an unambiguously defined physical interpretation. To demonstrate this, it is necessary to associate spectral line broadening and band shape to vibrational correlation functions. Raman spectra may be collected with the polarization axis defined parallel or perpendicular to the exciting beam. In this case, the depolarized scattering (perpendicular) spectrum $A_{\perp}(\omega) = A_{\text{anis}}(\omega)$ is determined entirely by the anisotropy of the polarizability tensor, whereas the polarized scattering (parallel) spectrum contains contributions from both isotropic and anisotropic components $A_{\parallel}(\omega) = A_{\text{iso}}(\omega) + \frac{4}{3}A_{\text{anis}}(\omega)$. Using this data, the isotropic Raman spectrum $A_{\text{iso}} = A_{\parallel}(\omega) - \frac{4}{3}A_{\perp}(\omega)$, normalized to the integrated spectral weight, may be related to the Fourier transform of the two-point vibrational correlation function $\langle q(0)q(t) \rangle$ as

$$A_{\text{iso}}(\omega) = \frac{A_{\parallel}(\omega) - \frac{4}{3}A_{\perp}(\omega)}{\int_{-\infty}^{\infty} (A_{\parallel}(\omega) - \frac{4}{3}A_{\perp}(\omega)) d\omega} \quad (2)$$

$$= \frac{1}{2\pi} \int_{-\infty}^{\infty} \langle q(0)q(t) \rangle \exp[i\omega t] dt, \quad (3)$$

where the ensemble average is taken over vibrational states.^{48,49} Thus, the isotropic Raman spectrum comprises only contributions from the vibrational part of the polarizability tensor.

For relaxations in the condensed phase, it is reasonable to assume that coupling to environmental degrees of freedom occurs through a Kubo–Anderson dephasing process. In this case, the vibrational transition frequency of an isolated oscillator ω_0 is modulated by a stochastic term $\omega_S(t)$ coupled to the molecular Hamiltonian $\hat{H} = \hat{H}_0 + \hat{H}_S$ through a perturbative correction $\hat{H}_S = \hbar\omega_S(t)$, where $\hat{H}_0$ is the Hamiltonian for the isolated system. Thus, the initial δ -function spectral band at ω_0 is broadened into a disseminated distribution about this peak through environmental coupling. Through a cumulant expansion,⁴² the correlation function for an oscillator undergoing such a dephasing exhibits a time dependence

$$\frac{\langle q(0)q(t) \rangle}{\langle |q(0)|^2 \rangle} = \exp[-\langle \omega^2 \rangle (\tau_c t - \tau_c^2 (1 - \exp[-t/\tau_c]))], \quad (4)$$

where the defining quantities are the frequency of the environmental perturbation τ_c^{-1} , such that $\langle \omega_S(0)\omega_S(t) \rangle \langle |\omega_S(0)|^2 \rangle^{-1} = \exp[-t/\tau_c]$, and the second moment of band contour

$$\langle \omega^2 \rangle = \frac{\int_{-\infty}^{\infty} A_{\text{iso}}(\omega) (\omega - \omega_0)^2 d\omega}{\int_{-\infty}^{\infty} A_{\text{iso}}(\omega) d\omega}. \quad (5)$$

Accordingly, the static frequency distribution of the system is determined by $\langle \omega^2 \rangle$ while the dynamical properties associated with environmental coupling are parameterized by the τ_c dependence. Adopting a single-parameter representation $\alpha = (\langle \omega^2 \rangle)^{1/2} \tau_c$ for the correlation function (Eq. (4)), expanding the inner exponent in a Taylor series, and performing a

Fourier cosine transform, the spectrum may be deconstructed into the functional form

$$K(\omega, \omega_0, \tau_c) = \frac{e^{\alpha^2}}{(\langle \omega^2 \rangle)^{1/2}} \sum_{n=0}^{\infty} \frac{(-\alpha^2)^n}{n!} \frac{\alpha + \frac{n}{\alpha}}{(\alpha + \frac{n}{\alpha})^2 + \frac{(\omega - \omega_0)^2}{\langle \omega^2 \rangle}} \quad (6)$$

corresponding to a symmetric band profile.^{42–44} The parameter α determines two regimes of spectral behavior. For a slow environmental perturbation ($\alpha \gg 1$), the spectral feature remains quasi-static, corresponding to the initial Gaussian distribution for the modulating frequency $\omega_S(t)$ with lineshape of width $\alpha/\tau_c = \langle \omega^2 \rangle^{-1/2}$. Conversely, in the limit of fast modulation ($\alpha \ll 1$), the vibrational spectrum is narrowed and assumes a Lorentzian lineshape of half-width $1/\Gamma = \langle \omega^2 \rangle \tau_c$ corresponding to the inverse dephasing time.⁴²

From a physical standpoint, it has been demonstrated that the perturbation time τ_c may be correlated with direct hydrogen bonding dynamics for a donor or acceptor, while the second band moment $\langle \omega^2 \rangle$ corresponds to the local environment of the associated functional group.⁴⁶ This suggests an analysis procedure in which the observed normalized isotropic spectrum $I_{\text{Iso,Obs}}(\omega_k)$ at wavenumber ω_k is subjected to a least squares fit

$$\chi^2 = \sum_{k=1}^N (I_{\text{Iso,Obs}}(\omega_k) - A_{\text{Iso}}(\omega_k))^2 \quad (7)$$

to a lineshape of the form

$$A_{\text{Iso}}(\omega) = \sum_{i=1}^N C_i K_i(\omega, \omega_0^i, \langle \omega^2 \rangle^i, \tau_c^i) + C_{\text{Solv}} A_{\text{Solv}}(\omega) + C_0, \quad (8)$$

where N is the number of constituent Kubo–Anderson functions, $K_i(\omega, \omega_0^i, \langle \omega^2 \rangle^i, \tau_c^i)$ are Kubo–Anderson functions themselves, $A_{\text{Solv}}(\omega)$ is the solvent background spectrum, and C_i , C_{Solv} , C_0 are constants. In this manner, it is possible to deconstruct a given observed spectrum into distinct molecular configurations corresponding to distinct solute-solvent interactions.

III. RESULTS AND DISCUSSION

A. Solvatochromic shifts

In prior investigations, the vibrational spectrum of benzophenone was assigned in solution and in the solid phase under a variety of physical conditions.^{30,50,51} While most Raman active modes of benzophenone exhibit no dependence on solvent structure, there are two features which are an exception to this rule. These modes comprise the carbonyl stretch, denoted $\nu(\text{C=O})$, which is found over a relatively broad region around $1662.8 \pm 7.2 \text{ cm}^{-1}$ and the aromatic stretching modes $\nu_i(\text{C-H})$ in the vicinity of $\sim 3066 \text{ cm}^{-1}$ (Fig. 1). The aromatic region is a poor probe of solvatochromic effects due to the presence of several closely spaced stretching bands, a comparatively weaker electric field dependence, and background contributions from several solvents in this region (see the supplementary material³⁴). Accordingly, the $\nu(\text{C=O})$ band affords the best monitor of local electrostatics within the benzophenone molecule.

FIG. 1. Raman spectra for benzophenone in CCl_4 highlighting the regions subject to solvatochromism (red), specifically the $\nu(\text{C}=\text{O})$ stretching mode (a) and the aromatic proton stretching region $\nu(\text{C}_{\text{ar}}-\text{H}_{\text{ar}})$ (b).

The practical detection limit for the benzophenone $\text{C}=\text{O}$ stretch in CH_2Cl_2 was found to be $100.0 \mu\text{M}$ with 441.6 nm excitation, however, the data acquisition time was increased from 2.0 min to 12.0 min to quantitate a meaningful spectrum. Nonetheless, this excitation wavelength is approximately 200 nm above the electronic absorption maximum at $\sim 250 \text{ nm}$ and hence these measurements are unlikely to benefit from any preresonance enhancement. Spectroscopic determinations at or near resonance would likely be enhanced 10- to 100-fold in terms of detection limit, indicating that the sensitivity of this Raman probe should surpass that of nitrile based labels under ideal conditions.²⁶ For the purpose of benchmarking $\nu(\text{C}=\text{O})$ solvatochromism, 100.0 mM benzophenone were adopted as a standard since this concentration affords an exceptional signal-to-noise ratio while avoiding major electrostatic self-interaction of benzophenone molecules. To quantify the solvatochromic response of benzophenone, it is necessary to adopt a metric for the effective electric field experienced by the molecule in solution. The simplest such estimate is afforded by the Onsager model.⁵² In this case, the solvent is treated as a continuum dielectric of constant ϵ in which a neutral solute is imbedded within a hollow, spherical cavity. A solute with gas phase electric dipole moment $\vec{\mu}$ will experience an effective electric field $\vec{E}_{\text{Ons}}$ according to

$$\vec{E}_{\text{Ons}}(\epsilon) = \frac{|\vec{\mu}|}{V} \left(\frac{2(\epsilon - 1)(n^2 + 2)}{3(2\epsilon + n^2)} \right), \quad (9)$$

where n and V are the index of refraction and cavity volume of the solute, respectively. For all calculations employing this model, a reference value was assumed for the index of refraction ($n = 1.6077$ at 19°C (Ref. 53)) the molecular volume obtained from the density of benzophenone ($\rho = 1.108 \text{ g cm}^{-3}$ (20°C); $V = 273.085 \text{ \AA}^3/\text{mol}$), and the dipole moment obtained from gas phase DFT calculations ($|\mu| = 3.0954 \text{ D}$; B3LYP/aug-cc-pVTZ^{35,54}). Conversion factors follow those utilized in prior calculations ($1 \text{ D} = 0.03336 \text{ aC \AA}$; $1 \text{ \AA}^3$ of polarization volume $= 0.01113 \text{ aC \AA}^2/\text{V}$; $1 \text{ cm}^{-1} = 1.9865 \times 10^{-5} \text{ aJ}$, $1.0 \text{ MV/cm} = 0.01 \text{ V/\AA}$).¹⁸

The vibrational shift of the $\nu(\text{C}=\text{O})$ band was observed in a series of solvents with varying dielectric strength, functional

groups, and steric properties (Fig. 2). In practice, the solvatochromic response of benzophenone is complex, with factors aside from electrostatics exerting influence over the $\nu(\text{C}=\text{O})$ shift. Solvents may be contextually grouped into three categories including general polar/nonpolar solvents (acetone, acetonitrile, cyclohexane, diethylamine, dimethyl sulfoxide (DMSO), dimethylformamide, ethyl acetate, tetrahydrofuran), halogenated or aromatic solvents (carbon tetrachloride, chloroform, dibromomethane, dichloromethane, toluene), and polar hydrogen bonding solvents (ethanol, methanol, 1-propanol, 2-propanol), with each category exhibiting distinct behavior.

Assuming that the only perturbation to $\nu(\text{C}=\text{O})$ is electrostatic, the Onsager model (Eq. (9)) indicates that the solvatochromic shift should vary with the reaction field as

FIG. 2. Observed dependence of the benzophenone solvatochromic Raman shift $\nu(\text{C}=\text{O})$ on the Onsager reaction field $|E_{\text{Ons}}|$ of the encapsulating solvent. Solvent shifts are grouped into four general categories including alcohol hydrogen bonding (yellow), alcohol bare shifts (green), halogen bonding/aromatic (blue/grey), and general polar/nonpolar solvents (red). The dashed regression line given by $\nu(\text{C}=\text{O}) = 3.530|E_{\text{Ons}}| + 1675.507$ is provided for the general solvent category ($R^2 = 0.93$).

$\Delta\nu = -\Delta\vec{\mu} \cdot \vec{E}_{\text{Ons}}/hc$. As expected, such a linear dependence is observed for general polar/nonpolar solvents with a significant degree of correlation (Fig. 2). The prominent role of electrostatics is not particularly surprising, as these solvents lack reactive functional groups and are unlikely to interact with benzophenone aside from as a dielectric background. Within this category, hexane affords the best model of benzophenone in vacuum due to its small dielectric constant and correspondingly weak reaction field. Conversely, DMSO provides the strongest reaction field explored for benzophenone while likewise containing no functional groups which are expected to interact directly with this solute. These two solvents are accordingly adopted as a reference scale for $\nu(\text{C}=\text{O})$ solvatochromism. The linear reaction field dependence of the general polar/nonpolar solvents follows that observed in implicit solvent DFT calculations, further underscoring the predictable behavior of this series (Fig. 3).

The halogenated solvents exhibit a marked departure from a linear reaction field effect. This family typically affords a solvatochromic shift twice that expected from a linear Onsager model, with the degree of departure decreasing as the ratio of protons to halogens in the solvent decreases (Fig. 2). Toluene also exhibits unusual behavior in this manner, which may be conceptually attributed to complexation with benzophenone in solution. To determine the source of this behavior, DFT calculations were performed in both implicit solvent and in the presence of a single solvent molecule

FIG. 3. Calculated Raman shift $\nu(\text{C}=\text{O})$ relative to hexane versus as a function of the Onsager reaction field $|E_{\text{Ons}}|$ arising from the solvent environment (B3LYP/cc-pVTZ). Shifts are grouped into categories corresponding to implicit solvent only for general polar/nonpolar solvents (red), implicit solvent for alcohols (green), explicit solvent with hydrogen bonding for alcohols (yellow), implicit halogenated solvent (dark blue), explicit halogenated solvent with halogen bonding (teal), and explicit halogenated solvent with hydrogen bonding (light blue). The dashed regression line given by $\nu(\text{C}=\text{O}) = 5.972|E_{\text{Ons}}| + 8.411$ represents a fit to all calculated implicit solvent points ($R^2 = 1.00$).

FIG. 4. Representative geometries for explicit solvent DFT calculations with halogenated solvents. Configurations include direct halogen (a) and hydrogen (b) contacts with the benzophenone carbonyl.

interacting with benzophenone with an implicit solvent background. The first set of models were constructed with the halogen or hydrogen atom on the solvent forming an explicit halogen or hydrogen bond to the benzophenone carbonyl. The intent of this series was to explore direct bonding effects (Fig. 4). In the second set, the halogen was placed above or alongside the aromatic ring of benzophenone in order to assess the feasibility of shifts in $\nu(\text{C}=\text{O})$ being induced by electronic perturbation of the ring system. Nonetheless, these complexes were found to dissociate and hence were neglected from further consideration. Similar behavior was observed for toluene complexes, indicating that a more detailed condensed phase treatment is necessary for this system. Explicit bonding to the $\text{C}=\text{O}$ was more physically realistic, with a dependence mirroring the experimental data in both the hydrogen-on and halogen-on configurations (Fig. 3). The hydrogen-on configurations afforded a larger shift in $\nu(\text{C}=\text{O})$ than the halogen-on configurations, however, a mixture of both bonding schemes may contribute in the condensed phase. Of these, the halogen bonding series more closely mimics experimental data, particularly when considering the aberrant hydrogen bonding shift calculated for CH_2Br_2 . This is notable since the carbon-halogen bond is more polar, and hence this effect would be expected to dominate hydrogen bonding in the halogenated solvent series.

The final discrepant category is the polar alcohols. In this case, the $\nu(\text{C}=\text{O})$ band is split into two separate spectral peaks. The high frequency peak is essentially conserved between the alcohols, suggesting a common origin for this behavior. Conversely, the location of the lower frequency peak is dependent on the solvent under consideration (Fig. 5). The splitting between these peaks is largest for the least hindered alcohol (methanol) and smallest for the most branched/hindered alcohol (2-propanol). It is tempting to ascribe this behavior to hydrogen bonding with the solvent. Such behavior is in fact observed in the values of $\nu(\text{C}=\text{O})$ predicted by DFT. In implicit solvent, the alcohols exhibit a similar value for the $\nu(\text{C}=\text{O})$ stretching frequency, which in turn fall within the range expected for a linear solvatochromic effect (Fig. 3). This is analogous to the experimentally observed high-frequency $\nu(\text{C}=\text{O})$ band. When explicit solvent is introduced in conjunction with implicit solvent background (Fig. 6), the hydrogen bond to $\nu(\text{C}=\text{O})$ shifts the band to

FIG. 5. Dependence of the benzophenone $\nu(\text{C}=\text{O})$ lineshape within primary and secondary alcohol solvents. Systems are plotted from top to bottom in order of decreasing Onsager reaction field $|\vec{E}_{\text{Ons}}|$.

lower vibrational frequencies, consistent with experimental data (Fig. 3). The presence of a split $\nu(\text{C}=\text{O})$ band differentiates the alcohols from the halogenated solvents, which exhibit a single perturbed peak. The halogenated solvents are expected to have a more rapid halogen/hydrogen bond interexchange in solution relative to the alcohols, a smaller solvent volume per molecule, and a greater number of orientations in which a single solvent molecule may interact with the carbonyl. A direct solvent–benzophenone interaction is then statistically more likely in the benzophenone/halogenated solvent system, and accordingly the isolated benzophenone may be undetectable experimentally due to its low abundance.

At first glance the vibrational shifts calculated from DFT afford at least qualitative agreement with experimental data. However, a detailed comparison indicates that the predictive value of these calculations is actually quite strong. A linear relationship exists between experimental and theoretical data

FIG. 6. Representative geometries for explicit solvent DFT calculations with alcohols. Configurations correspond to methanol (a), ethanol (b), 1-propanol (c), and 2-propanol (d).

FIG. 7. Cross correlation between calculated $\nu_{\text{DFT}}(\text{C}=\text{O})$ and observed $\nu_{\text{Obs}}(\text{C}=\text{O})$ values for the benzophenone $\text{C}=\text{O}$ stretch relative to hexane. Physically plausible series are represented by squares and comprise the general polar/nonpolar solvents (red), implicit solvent for alcohols (green), explicit solvent with hydrogen bonding for alcohols (yellow), explicit halogenated solvent with halogen bonding (teal), and explicit halogenated solvent with hydrogen bonding (light blue). Series which are not believed to be physically representative, specifically the implicit halogenated solvents and implicit toluene, are represented by grey crosses. The dashed line represents a regression $\nu_{\text{Obs}}(\text{C}=\text{O}) = 0.423\nu_{\text{DFT}}(\text{C}=\text{O}) - 2.7419$ for physically plausible series ($R^2 = 0.87$).

with $R^2 = 0.87$, suggesting a tight correlation (Fig. 7). Thus, while the calculated shifts are not identical to the experimental shifts, the use of reference data establish a correlation can provide a quantitative conversion factor. While prior studies avoided hydrogen bonding solvents due to difficulties in interpretation,⁹ these concerns may be mitigated when theoretical calculations are used to augment experimental investigations. As a final note, the scatter and deviation from a linear dependence of the solvatochromic shift on the Onsager reaction field may be attributed, in part, to the crude nature of the Onsager approximation. This limitation may be readily circumvented by employing classical or *ab initio* molecular dynamics simulations to explicitly determine either the electric field or the vibrational shift, respectively, of the probe with in the target medium.

B. Titration of H_2O : Benzophenone/ CH_3CN system

Titration experiments were performed using a reference system comprising a 1.87 M solution of benzophenone in acetonitrile. As a consequence of this high concentration, the Onsager reaction field approximation is inapplicable due to the contribution of solute–solute interactions. Accordingly, the analysis herein represents an approach to systems in which the solute itself contributes to the solvatochromic effect. Water was added to this system in increments of 0.2 equivalents to benzophenone, with isotropic Raman spectra acquired at each step to benchmark the evolution of the $\nu(\text{C}=\text{O})$ lineshape with increasing solvation (Fig. 8). Each spectrum corresponds to a distinct solvation environment for benzophenone. It is notable that both water and acetonitrile have completely featureless isotropic Raman spectra in the region of interest about the $\nu(\text{C}=\text{O})$ band, ranging between 1600 cm^{-1} and 1700 cm^{-1} .

FIG. 8. Titration of benzophenone in acetonitrile with water on an equivalent by equivalent basis as monitored by the $\nu(\text{C}=\text{O})$ band solvatochromic shift and lineshape. Pure benzophenone is plotted at top with increasing concentrations water in consecutive, descending plots.

Analysis of the anhydrous benzophenone/acetonitrile system indicates that the polarity of the mixture is substantially decreased relative to that employed in solvatochromic determinations due to the comparatively higher concentration of benzophenone. Accordingly, the $\nu(\text{C}=\text{O})$ marker band is shifted to 1660.6 cm^{-1} from a reference value of 1658.9 cm^{-1} in the more dilute 100 mM solution. As the concentration of water is increased, the center of $\nu(\text{C}=\text{O})$ peak undergoes a series of shifts, with distinct behavior observed at 1:1, 2:1, and 3:1 H_2O to benzophenone ratios (Table I). Beyond this point the frequency of the peak maximum ceases to shift and saturates at 1658.9 cm^{-1} , comparable to a more dilute benzophenone/acetonitrile mixture. The isotropic $\nu(\text{C}=\text{O})$ lineshape evolves on the same concentration scale as the band center, the FWHM initially narrowing with a 1:1 ratio H_2O of acetonitrile to water and progressively widening at higher ra-

TABLE I. General properties of the $\nu(\text{C}=\text{O})$ spectral feature for water: benzophenone/ CH_3CN solutions at several physically significant ratios.

$\text{H}_2\text{O:BP/CH}_3\text{CN}$	$\nu(\text{C}=\text{O})$ Maximum (cm^{-1})	$\nu(\text{C}=\text{O})$ FWHM (cm^{-1})
0:1	1660.6	13.2
1:1	1660.4	11.3
2:1	1659.5	13.0
3:1	1658.9	14.6
4:1	1658.9	15.3

FIG. 9. Representative geometries for the interaction of water with benzophenone in acetonitrile solutions. Configurations correspond to single (a) and double (b) molecule water chains in a hydrogen bonding network originating at the benzophenone carbonyl.

tios. Systems with a relative concentration greater than 4:1 were not explored due to the decreasing solubility of benzophenone and a concomitant increase in the opacity of the solution. Nonetheless, the saturation of both the peak shift and the band center suggests that no additional physical insight would be obtained by exploring higher concentrations of water.

The coincidence of major spectral shifts with integral equivalents of H_2O suggests that water complexes with benzophenone in solution. To explore this process in detail, the Kubo–Anderson dephasing model was employed to dissect lineshapes at a series of intermediate, solvated configurations. DFT calculations predict Raman shifts of -31.1 cm^{-1} and -34.7 cm^{-1} relative to hexane for single and double hydrogen bonding configurations, respectively (Fig. 9). Using this data, the calibration between experimental and theoretical spectra affords a separation between any two given hydrogen bonding configurations of less than 1.44 cm^{-1} . A two-component fit was employed with the assumption that the first component will correspond to benzophenone without hydrogen bonding and the second component to the collective ensemble of all hydrogen bonding configurations. Given that the small wavenumber separation between hydrogen bonding configurations is close to the single-to-noise limit for the spectrometer configuration, this approximation appears reasonable.

The Kubo–Anderson decomposition of $\nu(\text{C}=\text{O})$ spectra clearly delineates two regimes of solvated behavior (Fig. 10) with physically descriptive fit parameters (Table II). Benzophenone in anhydrous acetonitrile is characterized by a single spectral component centered at 1660.7 nm with $\alpha(1) < 1.0$, corresponding to a functional group relaxing through very transient or indirect interactions with the environment (Fig. 10). This description is consistent with a purely electrostatic perturbation of the $\nu(\text{C}=\text{O})$ band. As the ratio of water to benzophenone increases to 1:1, this band is shifted to lower wavenumbers through the solvatochromic effect. The peak motion may be attributed to the increasing dielectric strength of the solution due to the introduction of water. Concomitant with this shift is the development of a second subband characterized by a Kubo–Anderson function centered at 1645.5 cm^{-1} . At this point, α for the first subband increases slightly, while the second subband has a significantly larger value of $\alpha(2) > 1.0$, corresponding to a system with direct solvent interactions as expected for hydrogen bonding.

FIG. 10. Decomposition of the $\nu(\text{C}=\text{O})$ stretch in benzophenone into two Kubo–Anderson functions during titration of water into a benzophenone/acetonitrile solution. The two fitting functions are depicted alongside their sum. Experimental and fit band centers are presented outside and inside of parentheses, respectively.

The most reasonable configuration for this subband is a single water molecule hydrogen bonding to the benzophenone carbonyl and possibly the nitrile function of the solvent or the carbonyl of a second benzophenone molecule. The correlation time $\tau_c(2)$ for this band affords a measure of the mean weighted hydrogen bond lifetime for these configurations, in this case on the order of 7.7 ps.

Further increasing the concentration of water introduces a third regime of behavior for the $\nu(\text{C}=\text{O})$ band lineshape, with a distinct change apparent at a ratio of 2:1 waters per benzophenone molecule. Both subbands continue to shift to lower wavenumbers as a consequence of solvatochromism, while α increases slightly for the bare $\text{C}=\text{O}$ band and more substantially for the hydrogen bonding subband. More notably, the mean second band contour $\langle\omega^2(2)\rangle$ for the latter spectral feature decreases with a simultaneous increase in the correlation lifetime $\tau_c(2)$. Since the hydrogen bonding band is assumed to be an envelope containing contributions from all such configuration, this suggests the formation of a second family of hydrogen bonding arrangements. Such behavior is not unexpected, as the additional water molecules may form extended chains terminating on the carbonyl of benzophenone. The dynamic correlation time $\tau_c(2)$ likewise increases to 10.0 ps suggesting that hydrogen bonding networks of greater stability are formed with increasing solvation. At higher ratios of water to benzophenone, no further changes occur to either subband aside from a progressive solvatochromic shift of the band center to lower wavenumbers. Accordingly, the local solvation environment of benzophenone is likely determined by a max-

imum of two water molecules, presumably in a chain-like hydrogen bonding configuration (Fig. 9).

IV. CONCLUSIONS

While previous studies have calibrated solvatochromic effects against polar and nonpolar solvents, these studies have neglected more complex interactions such as hydrogen and halogen bonding.⁹ The results herein suggest that these effects are readily accommodated by augmenting experimental spectroscopy with electronic structure calculations, thereby affording a means to rationalize any deviation from purely electrostatic contributions. Coupled with Kubo–Anderson lineshape analysis this tool becomes even more versatile, facilitating exploration of the vibrational probe’s solvation shell. The successful use of QM/MM methods to calculate the perturbation of such a molecule within a protein environment attests to the potential of these techniques for exploration of biomolecular dynamics.¹¹

As demonstrated through the explorations herein, the choice of benzophenone as a Raman-active solvatochromic marker affords manifold advantages over established probes due to the strong spectral and electrostatic response of the carbonyl functional group. Furthermore, the potential of resonance Raman spectroscopy to enhance the detection limit of benzophenone suggests that it should surpass existing tools in terms of concentration sensitivity.²⁶ The primary utility of benzophenone as a vibrational probe would be found in the biological sphere. This chromophore has been incorporated into an unnatural amino acid, *p*-benzoyl-L-phenylalanine, which has been widely employed as a photoaffinity probe within this context.^{55,56} Consequently, procedures have been developed to incorporate and manipulate this amino acid within proteins.⁵⁷ A caveat does exist in that this probe cannot be utilized directly on resonance, per se, as the triplet state exhibits substantial radical pair reactivity following photoactivation at $\sim 350\text{--}360$ nm. The use of preresonance Raman spectroscopy at 405 nm, 406.7 nm, and 413.1 nm excitation wavelengths should circumvent this limitation. Nonetheless, this reactivity may be advantageous in cases where photoaffinity and solvatochromic probes are desired in the same system.

TABLE II. Kubo–Anderson fit parameters for titration of water into the benzophenone/ CH_3CN system. Units are specified for ω_0 in cm^{-1} , $\langle\omega^2(1)\rangle$ in cm^{-2} , and τ_c in ps. The index corresponding to the particular Kubo–Anderson fitting function is denoted in parentheses.

H ₂ O:BP	$\omega_0(1)$	$\langle\omega^2(1)\rangle$	$\tau_c(1)$	$\alpha(1)$	$\omega_0(2)$	$\langle\omega^2(2)\rangle$	$\tau_c(2)$	$\alpha(2)$
0:1	1660.7	60.9	4.0	0.9				
1:1	1660.4	61.4	4.5	1.1	1645.5	293.9	7.7	4.0
2:1	1659.4	53.2	7.1	1.6	1649.9	187.7	10.0	4.3
3:1	1658.8	55.8	7.1	1.6	1649.1	188.2	10.3	4.2
4:1	1658.7	56.1	7.0	1.6	1647.5	185.2	10.0	4.2

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