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Calculated Vibrational Properties of Ubisemiquinones

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Density functional theory has been used to calculate harmonic normal mode vibrational frequencies for unlabeled and isotope-labeled ubisemiquinones in both the gas phase and in several solvents. It is shown that four methoxy group conformations are likely to be present in solution at room temperature. Boltzmann weighted infrared and Raman spectra for the four conformers were calculated, and composite spectra that are the sum of the Boltzmann weighted spectra were produced. These composite spectra were compared to experimental FTIR and resonance Raman spectra, and it is shown that the calculated band frequencies, relative band intensities, and $^{13}$C and $^{18}$O isotope-induced band shifts are in excellent agreement with experiment. The calculations show that the C=O and C=C modes of ubisemiquinone strongly mix with methoxy methyl CH bending vibrations, and that the degree of mixing is altered upon isotope labeling, resulting in complicated changes in mode frequencies, intensities, and composition upon isotope labeling. Upon consideration of the calculated potential energy distributions of the normal modes of ubisemiquinone, and how they change upon isotope labeling, an explanation of some puzzling features in previously published Raman spectra is provided.

1. Introduction

Ubiquinones (UQ$_n$: 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinones) play an important role in biological electron and proton transfer processes that occur in both respiration and photosynthesis [1]. In photosynthetic reaction centers from purple bacteria, two UQ molecules, called Q$_A$ and Q$_B$, act as terminal electron acceptors [2]. In purple bacterial reaction centers (PBRCs) (see Abbreviations) from Rhodobacter (Rb.) sphaeroides, Q$_A$ and Q$_B$ are both ubiquinone-10 (UQ$_{10}$) molecules. Q$_A$ and Q$_B$ have very different functions; however, Q$_A$ is an intermediary cofactor involved in transferring electrons from bacteriopheophytin to Q$_B$, while Q$_B$ couples electron and proton transfer processes [3, 4]. The very different redox functions of Q$_A$ and Q$_B$ are testimony to the flexibility of UQs in biological processes. Since Q$_A$ and Q$_B$ are both UQ$_{10}$ molecules, pigment-protein interactions must modulate the functional properties of UQ$_{10}$ in PBRCs. Elucidation of these pigment-protein interactions is at the heart of much current research in photosynthesis [5, 6].

Fourier transform infrared (FTIR) difference spectroscopy (DS) is a sensitive molecular-level probe of pigment-protein interactions, and it is widely used to study both the neutral and reduced states of the quinones occupying the Q$_A$ and Q$_B$ binding sites in PBRCs [7]. Although Q$_A$ -/Q$_A$ and Q$_B$ -/Q$_B$ FTIR difference spectra have been obtained under a wide range of conditions for variously treated PBRCs, these spectra continue to be difficult to interpret because many bands not associated with the quinone also contribute to the spectra. Reconstitution of PBRCs with isotopically labeled quinones, however, has allowed some separation of the contributions of the quinones from those of the protein to the spectra [7]. Nonetheless the hypothesized band assignments in the experimental spectra, particularly those assignments associated with the ubiquinone anion radical, are still ambiguous and have not been modeled computationally.

One basis for developing an understanding of bands in Q$_A$ -/Q$_A$ and Q$_B$ -/Q$_B$ FTIR DS is to first consider spectra of the relevant quinones in solution. Infrared (IR) absorption spectra [8, 9] and resonance Raman spectra [10] for
ubisemiquinones in solution have been obtained. However, from a computational standpoint, even these simpler solution spectra are poorly understood. The work outlined in this paper is aimed at addressing this problem.

Few computational studies aimed at modeling the vibrational properties of ubisemiquinones (UQ\textsuperscript{−}) have been undertaken. The work that has been undertaken [11, 12] is limited in one way or another; for example, tail-less quinone models in only the gas phase were considered, using relatively low levels of theory. Previously it was claimed that the calculated normal modes and associated isotope-induced frequency shifts are in good agreement with experiment [12]. Isotope shifts do appear to agree with experiment. However, upon careful examination, it appears that the previously calculated normal modes (frequencies and intensities) are not in agreement with experimental spectra (see below). In the light of this finding we have used more robust computational methods to investigate the vibrational properties of ubisemiquinones in the gas phase and in solution.

In this paper we describe the simulation of FTIR and Raman spectra associated with labeled and unlabeled tail-containing ubisemiquinones in both the gas phase and in solvent.

2. Materials and Methods

2.1. Calculations. Molecular geometry optimizations and harmonic vibrational frequency calculations were performed using hybrid density functional theory (DFT) methods, employing the B3LYP functional and the 6-31 + G(d) basis set within Gaussian 03 [13]. 6-31 + G(d) is preferable to 6-31 G(d) for calculations involving semiquinones [14]. For calculations including solvent, the integral equation formalism (IEF) [15–17] of the polarizable continuum model (PCM) [18, 19] was used. The PCM uses the united atom cavity approach. Cavity parameters used were OFac = 0.89 (overlap index between interlocking spheres) and RS\textsubscript{min} = 0.2 (minimum radius in Angstroms for overlapping spheres). Very similar spectra were calculated when a smaller number of added spheres were considered (OFac = 0.8 and RS\textsubscript{min} = 0.5). The potential energy distribution (PED) (or total energy distribution) of normal modes was calculated using gar2ped [20].

Calculated normal mode vibrational frequencies presented here were scaled by 0.9808. Such a scale factor is standard for calculations using the specified functional and basis set and was derived by comparing the frequencies of bands in experimental and calculated spectra. Such a scaling of the calculated frequencies is undertaken only to facilitate a comparison between calculated and experimental spectra. We are primarily interested in vibrational frequency changes that occur upon isotope labeling, and these frequency differences are accurately calculated without scaling [14, 21].

3. Results

3.1. UQ Structure and Numbering. Figure 1 shows a geometry-optimized UQ\textsubscript{1−} model with the atom numbering scheme displayed. UQ has two carbonyl groups (C\textsubscript{1}=O\textsubscript{18} and C\textsubscript{4}=O\textsubscript{15}), two methoxy groups (C\textsubscript{3}=O\textsubscript{16}–CH\textsubscript{3} and C\textsubscript{2}=O\textsubscript{17}–CH\textsubscript{3}), a methyl group at C\textsubscript{5}, and an isoprene unit at C\textsubscript{6}. In our calculations we used UQ models with only a single isoprene unit. As outlined previously [21], the calculated vibrational properties of UQ\textsubscript{1−} (or UQ\textsuperscript{−}) are very similar to that found for UQ\textsubscript{n} (with n > 1). Also shown in Figure 1 are relevant internal coordinates of UQ\textsuperscript{−}. The normal modes will be expressed in terms of contributions from these internal coordinates. Of particular interest in this paper are the coordinates R\textsubscript{3}, R\textsubscript{9}, R\textsubscript{4}, and R\textsubscript{10} which are due to C\textsubscript{1}⋯O, C\textsubscript{4}⋯O, C\textsubscript{2}⋯C\textsubscript{5}, and C\textsubscript{6}⋯C\textsubscript{9} stretching vibrations, respectively. The methoxy methyl CH\textsubscript{3} bending vibrations (coordinates δ\textsubscript{C8} and δ\textsubscript{C9}) are also of considerable relevance in this paper, as they strongly couple to the C\textsubscript{2}⋯O vibrations (see below). This was not found to occur for neutral UQ [21].

3.2. Calculated Structure of Ubisemiquinone\textsubscript{1−} (UQ\textsubscript{1−}). Previously we showed that neutral UQ\textsuperscript{−} can adopt at least eight different methoxy group conformations at room temperature [21]. To establish which conformations may be present for UQ\textsuperscript{−}, single-point energy calculations were undertaken for methoxy group dihedral angles that were stepped in 10° increments. That is, 36 × 36 structures with fixed methoxy group dihedral angles were geometry optimized.

A contour plot of energy versus the C\textsubscript{2} and C\textsubscript{3} dihedral angles is shown in Figure 2, which indicates that there are four low-energy UQ\textsubscript{−} conformations, each with C\textsubscript{2} and C\textsubscript{3} dihedral angles close to ±120°. The four conformers are labeled A, B, E, and F in Figure 2. These four conformers are similar to the neutral UQ\textsuperscript{−} conformers labeled J, L, I, and K, respectively, that were described previously [21].

Following single-point energy calculations, the four UQ\textsubscript{1−} conformations were further geometry optimized (energy minimized) without constraining the dihedral angles. Calculations were undertaken for the four conformations in the gas phase and in several solvents that have dielectric constants spanning a wide range (2.2–78).

Calculated bond lengths, the C\textsubscript{6}–C\textsubscript{10}–C\textsubscript{11} bond angle, and methoxy group dihedral angles for the various UQ\textsubscript{1−} conformers in the gas phase and CCL\textsubscript{4} are listed in Table 1. Similar trends in the listed data are calculated for the conformers in other solvents (data not shown). Data for UQ\textsubscript{10}/UQ\textsubscript{10}– in the Q\textsubscript{A}/Q\textsubscript{B} binding site is also listed in Table 1.

The data presented in Table 1 demonstrates that all four conformers in solvent are within 0.45 kcal/mol in energy (kT at 298 K is ~0.59 kcal/mol), so all four conformers would be expected to be present to some degree in solvent at room temperature. The orientation of the methoxy groups of the four geometry-optimized conformers (in CCL\textsubscript{4}) as well as the calculated dihedral angles are shown in the insets in Figure 2. The corresponding dihedral angles for the four conformers in different solvents are similar (data not shown).

The hydrocarbon chain (isoprene unit) attached at C\textsubscript{6} makes a distinct kink at C\textsubscript{10}. The C\textsubscript{6}–C\textsubscript{10}–C\textsubscript{11} angle is close to 113° for all four conformers (Table 1). This angle is also
Figure 1: Structure and atomic numbering scheme for an optimized UQ\textsuperscript{−} model. Various internal coordinates are also outlined. R represents bond stretching, \(\alpha\) represents a bending of the angle between two bonds, and \(\delta\) represents a combination of angle bending centered at a vertex atom. For example, R4 represents a C=C\textsubscript{2} stretching vibration, \(\alpha_1\) represents a bending of the angle between the C\textsubscript{1}=C\textsubscript{2} and C\textsubscript{1}=C\textsubscript{6} bonds, and \(\delta C_8\) represents a bending vibration of the three C\textsubscript{8–H} groups.

Figure 2: Calculated optimized energy (in kcal/mol) of UQ\textsuperscript{−} for all C\textsubscript{2} (C\textsubscript{3}–C\textsubscript{2}–O\textsubscript{17}–C\textsubscript{9}) and C\textsubscript{3} (C\textsubscript{4}–C\textsubscript{3}–O\textsubscript{16}–C\textsubscript{8}) dihedral angles. The energy axis was shifted so that the lowest energy conformer was set to zero. The insets show the structures of the four optimized methoxy group conformers (obtained for calculations in CCl\textsubscript{4}). The emphasis is on displaying the methoxy group orientations, so hydrogen atoms have been removed and the tail at C\textsubscript{6} is not shown. Oxygen/carbon atoms are dark/light shade, respectively. C\textsubscript{2} and C\textsubscript{3} dihedral angles are also listed in each of the insets.
similar to that found for UQ$_{10}$/$UQ_{10}^-$ occupying the QA/QB binding site, respectively (Table 1).

### 3.3. Calculated Vibrational Frequencies of UQ$_4^-$

Figure 3(a) shows calculated IR spectra for the four UQ$_4^-$ conformers in CCl$_4$, in the 1530–1425 cm$^{-1}$ region. This spectral region is chosen because it is the region where the main C=C=O and C⋯C modes of UQ$^-$ lie, and it is therefore the region generally focused upon in FTIR studies of UQ$^-$ in solution [8–10]. The spectra of the conformers in Figure 3(a) have been scaled by the appropriate Boltzmann factors, which were calculated based on the relative energies of the four conformations (Table 1). A composite spectrum which is the sum of the four Boltzmann weighted spectra is also shown in Figure 3(a). The corresponding calculated composite spectra for UQ$^-$ in various solvents are presented in Figures 3(b) and 3(c).

In the composite spectra an intense band is observed at 1500–1478 cm$^{-1}$, depending on the solvent. The frequency of this absorption band decreases, and the intensity increases, as the dielectric constant of the solvent increases. The frequency changes as a function of dielectric constant are outlined in the inset in Figure 3(a), which demonstrates that the band frequency is strongly solvent dependant only for solvents with dielectric constant ranging from ~1 to 20. Similar results have been found for PCM calculations of small neutral ketones in nonprotic solvents [22].

The calculated composite spectrum for UQ$_4^-$ in CCl$_4$ (Figure 3(a)) displays an intense band at 1493 cm$^{-1}$. Lower intensity peaks are observed at 1483 and 1450 cm$^{-1}$. Table 2(b) lists the frequencies, IR intensities, Raman activities, and potential energy distributions for the normal modes that contribute to the bands in the spectra of UQ$_4^-$ in CCl$_4$. Similar results are calculated for conformers A, B, and E (data not shown), as would be expected given the similarity in the spectra of the conformers in Figure 3(a). For comparison, Table 2(a) also lists data for UQ$_{10}^-$ in the gas phase.

For UQ$_{10}^-$ in CCl$_4$, the band at 1493 cm$^{-1}$ (Figure 3(a)) is due to two intense normal modes at ~1491 and ~1495 cm$^{-1}$. The 1491 cm$^{-1}$ normal mode is due predominantly to C=C=O stretching [R9(56%)] while the 1491 cm$^{-1}$ normal mode is due predominantly to C=C=O stretching [R3(46%)]. For all four conformers, the C=C=O and C⋯C groups vibrate separately at a similar frequency with similar intensity. This is also observed for UQ$_{10}^-$ in other solvents (not shown).

In all spectra in Figure 3 a weak band is found at 1522 cm$^{-1}$. This band is due to an out-of-phase vibration of the C⋯C groups of the quinone ring (R4–R10). Given that the antisymmetric nature of the vibration it is very weakly Raman active. The in-phase vibration of the C⋯C groups of the quinone ring occurs at 1607 cm$^{-1}$ and is IR silent but very strongly Raman active (Table 2).

A relatively intense band is found at 1450–1456 cm$^{-1}$ in all of the spectra in Figure 3. This band is due predominantly to CH bending vibrations of both methoxy methyl groups (δC8 and δC9) (Table 2(b)). Given that the mode is due to CH bending vibrations of the methoxy methyl groups it is not surprising that the precise frequency of this normal mode can vary by as much as 5 cm$^{-1}$ among the four conformers (Figure 3(a)).

The calculated spectra of the four UQ$_4^-$ conformers are similar (Figure 3(a)). We also find that the spectra are very similar for isotope-labeled versions of the conformers (not
shown). For this reason we will consider only the Boltzmann weighted composite spectra below. In addition, we will consider spectra for UQ$_{1}^{-}$ in CCl$_{4}$, noting that similar results and conclusions hold for UQ$_{1}^{-}$ in other solvents.

Figure 4 shows calculated IR (left) and Raman (right) spectra for unlabeled, $^{13}$C, and $^{18}$O isotope-labeled UQ$_{1}^{-}$ in the gas phase (a) and CCl$_{4}$ (b). The normal modes (frequencies, intensities, Raman activities, and PEDs) that give rise to the bands in the spectra in Figure 4 are also listed in Table 2.

As discussed above, for unlabeled UQ$_{1}^{-}$ in CCl$_{4}$ the 1493 cm$^{-1}$ band (IR spectrum) is due to separate C$_{4}$$\cdots$O and C$_{1}$$\cdots$O vibrations. Upon $^{13}$C labeling the 1493 cm$^{-1}$ band appears to downshift 39 cm$^{-1}$ to 1454 cm$^{-1}$ (Figure 4(b)). Such a downshift is expected for a band that is due to C$_{4}$$\cdots$O groups. Table 2(b) indicates that the 1454 cm$^{-1}$ band in the spectrum of $^{13}$C labeled UQ$_{1}^{-}$ in CCl$_{4}$ is due to a C$_{4}$$\cdots$O stretching vibration mixed with CH methyl bending vibrations (associated with both methoxy methyl groups). A very low-intensity normal mode at 1458 cm$^{-1}$ also contributes to the 1454 cm$^{-1}$ band in the IR spectrum of $^{13}$C-labeled UQ$_{1}^{-}$ in CCl$_{4}$. This 1458 cm$^{-1}$ mode is due to a C$_{1}$$\cdots$O stretching vibration mixed with CH methoxymethyl bending vibrations (Table 2(b)). So both C$_{4}$$\cdots$O groups give rise to intense normal modes for unlabeled UQ$_{1}^{-}$ in CCl$_{4}$. However, upon $^{13}$C labeling, only one intense C$_{4}$$\cdots$O mode is found while the other is considerably weaker. Similar $^{13}$C isotope-induced changes are found for calculations of UQ$_{1}^{-}$ in the gas phase (Table 2(a)).

The band at 1449 cm$^{-1}$ in the IR spectrum for unlabeled UQ$_{1}^{-}$ in CCl$_{4}$ is due predominantly to CH bending vibrations of both methoxy methyl groups. Upon $^{13}$C labeling the 1449 cm$^{-1}$ band downshifts from 18 cm$^{-1}$ to 1431 cm$^{-1}$ (Figure 4(b)). The 1431 cm$^{-1}$ mode is due to the out-of-phase vibration of both C$_{4}$$\cdots$O groups [$-R3(24\%) + R9(11\%)] coupled to a C$_{4}$ methoxymethyl bending vibration [688(32\%)]. Other than the normal modes just discussed, C$_{4}$$\cdots$O stretching vibrations (R9 and R3) contribute to at least 6 other modes in the 1500–1400 cm$^{-1}$ region for $^{13}$C-labeled UQ$_{1}^{-}$. Similar results are found for $^{13}$C-labeled UQ$_{1}^{-}$ in the gas phase.

In the IR spectrum of unlabeled UQ$_{1}^{-}$ in CCl$_{4}$ the weak band at 1523 cm$^{-1}$ is due predominantly to an out-of-phase C$_{4}$$\cdots$C vibration (R4–R10). The in-phase C$_{4}$$\cdots$C vibration (R4 + R10) occurs at 1607 cm$^{-1}$, with negligible IR intensity but high Raman activity (Figure 4(b)). The in-phase C$_{4}$$\cdots$C mode downshifts 57 cm$^{-1}$ to 1560 cm$^{-1}$ upon $^{13}$C labeling with little change in the mode composition (Table 2(b)).

In the unlabeled species the relatively pure C$_{4}$$\cdots$O modes are found near 1493 cm$^{-1}$ (in CCl$_{4}$). Upon $^{18}$O labeling these C$_{4}$$\cdots$O modes are expected to downshift from $\sim$40 cm$^{-1}$ to $\sim$1453 cm$^{-1}$. In the unlabeled species the methoxy-methyl bending mode is found at 1450 cm$^{-1}$. So upon $^{18}$O labeling the C$_{4}$$\cdots$O modes and methyl bending modes will be similar in frequency and are therefore expected to strongly mix.

**Figure 3:** (a) Calculated Boltzmann weighted IR spectra for the four UQ$_{4}^{-}$ conformations in CCl$_{4}$: UQ$_{4A}^{-}$ and UQ$_{4B}^{-}$ (dotted), UQ$_{4C}^{-}$ and UQ$_{4D}^{-}$ (solid). A composite spectrum that is the sum of the Boltzmann weighted spectra is also shown (thick line). (b) Calculated composite IR spectra for UQ$_{1}^{-}$ in the gas phase (black), CCl$_{4}$, MeCN (red), THF (blue), DCM (magenta), and acetone (purple). (c) Calculated composite IR spectra for UQ$_{1}^{-}$ in ethanol (purple), methanol (black), MeCN (red), DMSO (blue), and H$_{2}$O (magenta). The spectra displayed with thin lines in B/C are the spectra from C/B, respectively. All frequencies were scaled by 0.9808. **Inset:** graph of peak frequency as a function of solvent dielectric constant. Dielectric constants for CCl$_{4}$, THF, DCM, acetone, ethanol, methanol, acetonitrile, DMSO, and H$_{2}$O are 2.23, 7.58, 8.93, 20.7, 24.3, 32.63, 36.64, 46.7, and 78.39, respectively.
Table 2: Calculated vibrational frequencies (in cm$^{-1}$), intensities (in km/mol), Raman activities (in Å$^4$/amu), and potential energy distributions (%) of normal modes that contain contributions from C⋯O and/or C⋯C groups (R3, R9, R4, and R10) of unlabeled, $^{13}$C-, and $^{18}$O-labeled UQ$_1$ in (a) the gas phase and (b) CCl$_4$. Only contributions to the PED above 5% are considered. Frequencies are scaled by a factor 0.9808. For $^{18}$O isotope labeling only the carbonyl oxygen atoms are labeled. $\nu_i$ = $i$th bond stretching; $\delta C_i$ = $X$–$C_i$–$X$ bending for –CH$_3$, –CH$_2$– and –CH= groups; $X$ = atom bonded to $C_i$; $RD_1 = 6^{-1/2}(\alpha_1 – \alpha_2 + \alpha_3 – \alpha_4 + \alpha_5 – \alpha_6)$; $RD_2 = 12^{-1/2}(2\alpha_1 – \alpha_2 – \alpha_3 + 2\alpha_4 – \alpha_5 – \alpha_6)$ = ring deformation; $\alpha_i$ = $C_{i-1}$–$C_i$–$C_{i+1}$ angle bending of ring atoms.

(a) Gas phase

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(b) CCl$_4$

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<td>1607</td>
<td>11</td>
<td>869</td>
<td>R4(27) + R10(23) + RD2(12)</td>
</tr>
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<td></td>
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<td>$^{13}$C</td>
</tr>
<tr>
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<td>51</td>
<td>33</td>
<td>$\delta C7$(79) + R11(7) + R9(5)</td>
</tr>
<tr>
<td>1421</td>
<td>21</td>
<td>42</td>
<td>$\delta C9$(28) + $\delta C8$(18) – R10(13) + R4(11) + R3(9)</td>
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<tr>
<td>1431</td>
<td>365</td>
<td>7</td>
<td>$\delta C8$(32) – R3(24) + R9(11)</td>
</tr>
<tr>
<td>1443</td>
<td>15</td>
<td>91</td>
<td>$\delta C9$(39) + R10(14) – R9(12) – R3(11) + $\delta C7$(6)</td>
</tr>
</tbody>
</table>
Table 2(b) shows that upon $^{18}$O labeling the \( \text{C} \cdots \text{O} \) stretching vibrations do mix extensively with methyl bending vibrations, and that the \( \text{C} \cdots \text{O} \) modes are distributed amongst at least five different mixed modes. From the IR spectra in Figure 4(b), one could argue that the 1493 cm\(^{-1}\) band downshifts from 18 cm\(^{-1}\) to 1475 cm\(^{-1}\) upon $^{18}$O labeling. Such an $^{18}$O isotope-induced frequency shift for semiquinones is in line with experimental observations [8, 23] (see below).

4. Discussion

The calculated changes in frequency, intensity, and mode composition upon isotope labeling of ubisemiquinone are considerably more complex than those found for the neutral species [21]. In spite of this, however, the calculated data allow a clear and detailed interpretation of bands in experimental Raman and FTIR spectra of ubisemiquinone. The calculated IR and Raman spectra presented in Figure 4(b) correspond very well to experimental spectra [8–10].

### 4.1. Modeling Isotope-Induced Bandshifts Observed in Resonance Raman Spectra.

Resonance Raman spectra of unlabeled and $^{13}$C-labeled UQ\( _{10} \) in the QA and QB binding sites in purple bacterial reaction centers have been obtained [10]. For comparison, resonance Raman spectra of unlabeled UQ\( _{10} \) in solution were also obtained [10]. For both in vivo and in vitro cases an intense Raman band was observed near 1608 cm\(^{-1}\), with weaker bands observed near 1523 and 1488 cm\(^{-1}\). We note that the calculated Raman spectrum for unlabeled UQ\( _{10} \) (Figure 4(b)) looks very similar to the experimental spectrum.

The $\sim$1608 cm\(^{-1}\) band was assigned to a \( \text{C} \cdots \text{C} \) mode, weakly coupled to a \( \text{C} \cdots \text{O} \) mode, because it downshifted to 49–58 cm\(^{-1}\) upon $^{13}$C labeling [10] (Table 3). The $\sim$1488 cm\(^{-1}\) band was assigned to a \( \text{C} \cdots \text{O} \) mode because it downshifted to $\sim$28 cm\(^{-1}\) upon $^{18}$O labeling [10] (Table 3). The $\sim$1523 cm\(^{-1}\) band apparently disappears upon $^{13}$C labeling. Although not suggested in the original manuscript, it is possible that the 1523 cm\(^{-1}\) band (of UQ\( _{10} \))
binding site) downshifts from $\sim 67 \text{ cm}^{-1}$ to 1456 cm$^{-1}$ upon $^{13}$C labeling, and is masked by the $^{13}$C⋯$^{18}$O band at 1456 cm$^{-1}$ (Table 3).

In our calculations the C$_2$⋯C$_3$ and C$_5$⋯C$_6$ stretching vibrations couple to give C⋯C in-phase and out-of-phase vibrations. For UQ$^1_{-1}$ in CCl$_4$, the C⋯C in-phase vibration is at 1607 cm$^{-1}$ (Figure 4(b)). This is 84 cm$^{-1}$ higher in frequency than the out-of-phase vibration (at 1523 cm$^{-1}$).

Unlike the out-of-phase vibration, the in-phase vibration is not coupled with methyl bending and carbonyl stretching modes (Table 2(b)). The C⋯C in-phase vibration has negligible IR intensity but huge Raman activity. It is basically unaffected by $^{18}$O labeling, but downshifts to 57 cm$^{-1}$ upon $^{13}$C labeling. The intensely Raman active band calculated at 1607 cm$^{-1}$ for UQ$^1_{-1}$ clearly corresponds to the band observed at $\sim 1608 \text{ cm}^{-1}$ experimentally [10].

**Figure 4**: Calculated Boltzmann weighted composite IR (left) and Raman (right) spectra for unlabeled (top), $^{13}$C (middle), and $^{18}$O (bottom) labeled UQ$^1_{-1}$ in (a) the gas phase and (b) CCl$_4$. For $^{18}$O isotope labeling only the carbonyl oxygen atoms are labeled.
The out-of-phase C\textsubscript{\textdegree}C vibrational mode at 1523 cm\textsuperscript{-1} mixes with other modes upon \textsuperscript{13}C labeling and is not easily identifiable. A strongly Raman active mode of \textsuperscript{13}C-labeled UQ\textsubscript{10} is calculated at 1458 cm\textsuperscript{-1} (Table 2(b)). The out-of-phase C\textsubscript{\textdegree}C vibration contributes 17\% to the PED of this mode [R10(6\%)–R4(11\%)]. The in-phase-coupled vibration of both C\textsubscript{\textdegree}/C\textsubscript{\textdegree}O groups [R3(15\%) + R9(7\%)] also contributes to this mode.

Clearly, the calculated out-of-phase C\textsubscript{\textdegree}C vibrational mode at 1523 cm\textsuperscript{-1} can be associated with the band observed at \sim 1521 cm\textsuperscript{-1} in resonance Raman spectra of UQ\textsuperscript{−} in solution [10]. We suggest that the mode calculated at 1523 cm\textsuperscript{-1} forms part of a new mode that appears at 1458 cm\textsuperscript{-1} upon \textsuperscript{13}C labeling (Table 3). In phase \textsuperscript{13}C\textsubscript{\textdegree}/C\textsubscript{\textdegree}O vibrations also contribute to the 1458 cm\textsuperscript{-1} mode. Our calculated data therefore provides an explanation as to why the \sim 1521 cm\textsuperscript{-1} resonance Raman band that is observed experimentally is not identified in spectra of \textsuperscript{13}C-labeled UQ\textsuperscript{−} [10]. Upon \textsuperscript{13}C labeling the C\textsubscript{\textdegree}C mode mixes with C\textsubscript{\textdegree}/O modes (and methyl bending modes) to become a new mode that is not distinctly identifiable as a \textsuperscript{13}C\textsubscript{\textdegree}/\textsuperscript{13}C\textsubscript{\textdegree}C mode.

Bands at 1486/1489 cm\textsuperscript{-1} in resonance Raman spectra of UQ\textsuperscript{−} in the QA/QB binding site downshifts 30/27 cm\textsuperscript{-1} upon \textsuperscript{13}C labeling of UQ\textsuperscript{−} (Table 3), respectively. They were therefore associated with C\textsubscript{\textdegree}/C\textsubscript{\textdegree}O modes coupled to C\textsubscript{\textdegree}C modes. Computationally, we find two C\textsubscript{\textdegree}C/O modes at 1495 and 1491 cm\textsuperscript{-1}. Both modes are Raman active with the 1491 cm\textsuperscript{-1} mode displaying the greater activity (Table 2(b)). These modes give rise to the 1492 cm\textsuperscript{-1} band in the calculated Raman spectrum (Figure 3(b)), which appears to downshift 19/35 cm\textsuperscript{-1} to 1473/1457 cm\textsuperscript{-1} upon \textsuperscript{13}C labeling. The 1473/1457 cm\textsuperscript{-1} band in the calculated Raman spectrum for \textsuperscript{13}C labeled UQ\textsubscript{10\textsuperscript{−}} is dominated by a mode at 1474/1458 cm\textsuperscript{-1}, respectively. The 1474 cm\textsuperscript{-1} mode and to a lesser degree the 1491 cm\textsuperscript{-1} mode are due predominantly to methyl CH bending vibrations of both methoxy groups coupled to a C\textsubscript{\textdegree}/O vibration. Notice that the coupling of the C\textsubscript{\textdegree}/C\textsubscript{\textdegree}C vibration is to methoxy methyl CH bending vibrations, not C\textsubscript{\textdegree}/C\textsubscript{\textdegree}C ring vibrations, as was originally proposed based on the experimental spectra.

4.2. Modeling Isotope-Induced Bandshifts Observed in FTIR Spectra. Electrochemically generated FTIR difference spectra of UQ in various solvents have been obtained [8]. For UQ\textsubscript{10\textsuperscript{−}} in acetonitrile, THF, or dichloromethane an intense FTIR absorption band was observed at 1483–1488 cm\textsuperscript{-1}. The observation of a predominantly a single intense band in experimental FTIR spectra of unlabeled UQ\textsubscript{10\textsuperscript{−}} and UQ\textsubscript{18\textsuperscript{−}} in solution is in line with our calculated IR spectra, which are dominated by an intense band at 1478–1493 cm\textsuperscript{-1} for UQ\textsubscript{18\textsuperscript{−}} in a variety of solvents (Figures 3(b) and 3(c)).

In experimental FTIR difference spectra for UQ\textsubscript{18\textsuperscript{−}} in dichloromethane, a band is observed at 1483 cm\textsuperscript{-1}, which downshifts to 41 cm\textsuperscript{-1} upon \textsuperscript{13}C labeling (Table 3) [8]. From Figure 3(b) it can be seen that upon \textsuperscript{13}C labeling the 1493 cm\textsuperscript{-1} band downshifts from 39 cm\textsuperscript{-1} to 1454 cm\textsuperscript{-1}. The calculated result therefore agrees very well with the experimental observation.

Experimentally, for UQ\textsubscript{18\textsuperscript{−}} in dichloromethane, it is also observed that the 1483 cm\textsuperscript{-1} band downshifts from 15 cm\textsuperscript{-1} to 1468 cm\textsuperscript{-1} upon \textsuperscript{18}O labeling. From the calculated IR spectra in Figure 4(b), the most obvious suggestion is that the 1493 cm\textsuperscript{-1} band (of unlabeled UQ\textsubscript{18\textsuperscript{−}}) downshifts from 18 cm\textsuperscript{-1} to 1475 cm\textsuperscript{-1} upon \textsuperscript{18}O labeling. The calculated PEDs in Table 2(b) indicate a complicated situation: the 1495 and 1491 cm\textsuperscript{-1} modes of unlabeled UQ\textsubscript{18\textsuperscript{−}} are due to the C\textsubscript{\textdegree}/O groups (R3 and R9, resp.). Upon \textsuperscript{18}O labeling modes appear at 1476 [R9(23\%)] and 1465 cm\textsuperscript{-1} [R9(27\%)]. Thus, the 1491 cm\textsuperscript{-1} mode in the unlabeled species appears to split and downshift to 15 and 26 cm\textsuperscript{-1} upon \textsuperscript{18}O labeling (Table 3). The former is in excellent agreement with experiment [8]. Upon \textsuperscript{18}O labeling modes also appear at 1481 [R3(14\%)] and 1483 cm\textsuperscript{-1} [R3(7\%)]. Thus, the 1495 cm\textsuperscript{-1} mode in the unlabeled species also appears to split and downshift to 14 and 12 cm\textsuperscript{-1} upon \textsuperscript{18}O labeling (Table 3). Again, these conclusions are in good agreement with experiment [8]. It is the plethora of mixed modes that appear upon \textsuperscript{18}O labeling that give rise to the broad band with a peak near 1475 cm\textsuperscript{-1} in the calculated spectrum (Figure 3(b)). Unfortunately FTIR spectra for \textsuperscript{18}O-labeled UQ\textsubscript{18\textsuperscript{−}} have never been presented. Only the observed shifts upon labeling were presented.

From electrochemically generated FTIR difference spectra of \textsuperscript{13}C-labeled UQ\textsubscript{10\textsuperscript{−}} in various solvents [8] a band was observed at 1412 cm\textsuperscript{-1}. It was suggested that this band was due to a \textsuperscript{13}C\textsubscript{\textdegree}/\textsuperscript{13}C\textsubscript{\textdegree}C vibration that was downshifted to 71 cm\textsuperscript{-1} from 1483 cm\textsuperscript{-1} in the unlabeled species. Neither the calculated data presented here nor the resonance Raman data presented previously support this hypothesis.

4.3. Experimental QA\textsuperscript{−}/QA and QB\textsuperscript{−}/QB FTIR DS. QA\textsuperscript{−}/QA and QB\textsuperscript{−}/QB FTIR DS have been obtained using PBRCs from R. sphaeroides [7, 24–27]. In QA\textsuperscript{−}/QA FTIR DS three intense IR bands are observed near 1485, 1466, and 1449 cm\textsuperscript{-1} [24, 25]. On the basis of \textsuperscript{18}O, \textsuperscript{13}C, \textsuperscript{13}C\textsubscript{\textdegree}, and \textsuperscript{13}C\textsubscript{\textdegree} vibration the 1486/1466 cm\textsuperscript{-1} bands were assigned to C\textsubscript{\textdegree}/C\textsubscript{\textdegree}O vibrations, respectively [24]. The modes were suggested to be considerably mixed. The origin of the 1449 cm\textsuperscript{-1} band was not considered.

Another group, which undertook identical labeling experiments [25], assigned the 1485 cm\textsuperscript{-1} band to a C\textsubscript{\textdegree}/O vibration, the 1466 cm\textsuperscript{-1} band to C\textsubscript{\textdegree}=O vibration, and the 1449 cm\textsuperscript{-1} band to a C\textsubscript{\textdegree}/C\textsubscript{\textdegree}C vibration. All modes were suggested to be strongly mixed.

Resonance Raman spectra for UQ\textsuperscript{−} in the QA binding site display a weak band at 1486 cm\textsuperscript{-1}, but no bands were apparent at 1466 and 1449 cm\textsuperscript{-1}. Of course it may simply be the case that the 1466 and 1449 cm\textsuperscript{-1} normal modes are Raman inactive.

Our calculated spectra for UQ\textsubscript{18\textsuperscript{−}} in solution poorly model observed FTIR bands of UQ\textsuperscript{−} in the QA binding site. For UQ\textsuperscript{−}
in the QA binding site, the C···O modes appear to be separated by 19 cm\(^{-1}\). For calculations in solvent the two C···O modes do appear to be distinct, although the separation of the modes is only 4 cm\(^{-1}\). In gas phase calculations the two C···O modes are coupled. The separation of C···O modes of UQ\(^{-}\) in the QB binding site is due to asymmetric interactions with the protein environment. Calculations of UQ\(^{-}\) in solvent or in the gas phase cannot model these interactions. Calculations including effects of the protein environment are essential. Such calculations are underway in our lab.

In Q\(_{B}\) /Q\(_{B}\) FTIR DS a single IR band is observed near 1479 cm\(^{-1}\). It was suggested that this band was due to both C···O modes of UQ\(^{-}\) in the QB binding site [7, 26, 27]. It was also suggested that the 1479 cm\(^{-1}\) band downshifts 33/52 cm\(^{-1}\) upon \(^{18}\)O/\(^{13}\)C labeling, respectively [7, 26, 27]. Such shifts are difficult to rationalize in view of the shifts calculated (15/37 cm\(^{-1}\)) and observed experimentally (15/27–41 cm\(^{-1}\)) for UQ in solution (Table 3). Additionally, there appears to be some inequivalence in the C···O modes of UQ in the QB binding site when perturbed specifically at the C\(_{5}\) or C\(_{6}\) position [7, 26, 27]. It was suggested that this inequivalence is a result of specific protein interactions [7, 26, 27]. Again, calculations including effects of the protein environment appear to be necessary (essential) in order to accurately simulate the vibrational spectra of UQ in the QB binding site.

### 4.4. Previous Calculations of Ubisemiquinones

DFT-based vibrational frequency calculations (using the BP86 functional) have been undertaken for 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxynaphthalene in the gas phase [12]. Comparison of calculated data for the two models showed that substituents at C\(_{5}\) and C\(_{6}\) are required in order to better model the properties of ubiquinones and ubisemiquinones. In the above study isotope shifts were calculated. However how the C···O and C···C modes couple with each other and with CH methoxy methyl bending vibrations was not considered. As we have shown above, the extent of mode mixing can be considerably altered upon labeling, making it difficult to identify how the different bands shift upon labeling. As we show here, the detailed PEDs are a crucial tool in the analysis of how calculated bands shift upon isotope labeling.

One problem with previous DFT calculations (in the gas phase) [12] is that for 2,3-dimethoxy-1,4-benzoquinone the C···O modes were found at a higher frequency than the C···C modes. For 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone (in the gas phase) the C···O modes were found at slightly lower frequency than the C···C modes (3-4 cm\(^{-1}\)). However, from Raman experiments the out-of-phase C···C mode is found to be \(\sim 32\) cm\(^{-1}\) higher in frequency than the C···O mode [10] (Table 3).

Furthermore, the antisymmetrically coupled C···O mode (for 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone) was calculated to be more than a factor of 26 times more intense than the C···C mode [12]. This calculated result is not in line with experimental IR spectra [8].

Clearly, previous DFT calculations [12] poorly model the experimental Raman and IR spectra. In contrast, in our calculations for UQ\(_{1}\) in CCl\(_{4}\), the out-of-phase C···C modes are 28–32 cm\(^{-1}\) higher in frequency than either of the C···O mode (Table 2(b)). In gas phase calculations the out-of-phase C···C mode is still 24 cm\(^{-1}\) higher in frequency than the antisymmetrically coupled C···O mode (Table 2(a)). In addition, in gas phase calculations and in solvent, the intensity of antisymmetrically coupled C···O mode is \(\sim 7.5\) times more intense than the out-of-phase C···C mode. These results are in excellent agreement with experimental IR and Raman spectra. The limitations in previous calculations are most likely related to the choice of functional and basis set, and the inadequacy of a UQ structural model that lacks an isoprene unit.

### 5. Conclusions

We calculate that four UQ\(_{1}\) conformers are likely present in solution at room temperature. Calculated IR spectra for all four UQ\(_{1}\) conformers are similar. Calculated IR spectra of unlabeled and isotope-labeled UQ\(_{1}\) in the gas phase and in solution show a similar band pattern, although in some cases there are differences in the composition of the modes that contribute to the bands in the spectra.

Calculations show that upon isotope labeling the out-of-phase C···C ring modes and C···O modes of UQ\(_{1}\) strongly couple with methyl C–H bending vibrations of the methoxy groups. This leads to complicated splitting of modes and unusual downshifts upon isotope labeling. Nonetheless by consideration of PEDs of the calculated normal modes, sense can be made of the isotope-induced shifts and intensity changes, and it is shown that the calculated data provide a rational and detailed interpretation of experimentally observed isotope-induced band shifts in experimental FTIR and Raman spectra of UQ\(_{1}\) in solution.

### Abbreviations

- **DFT**: Density functional theory
- **DS**: Difference spectra
- **FTIR**: Fourier transform infrared
- **IR**: Infrared
- **IEF**: Integral equation formalism
- **PBRCs**: Purple bacterial reaction centers
- **PCM**: Polarizable continuum model
- **PED**: Potential energy distribution
- **UQ**: Ubiquinone
- **UQ\(_{1}\)**: Ubisemiquinone.

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References


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