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**UNIVERSITY OF OKLAHOMA
GRADUATE COLLEGE**

**A COMPUTATIONAL STUDY OF THE STRUCTURAL, SPECTROSCOPIC,
THERMOCHEMICAL AND BINDING PROPERTIES OF QUINONES
INVOLVED IN PHOTOSYNTHESIS**

**A Dissertation
SUBMITTED TO THE GRADUATE FACULTY
in partial fulfillment of the requirements for the
degree of
Doctor of Philosophy**

**By
Anthony Kurt Grafton
Norman, Oklahoma
1998**

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**A COMPUTATIONAL STUDY OF THE STRUCTURAL, SPECTROSCOPIC,
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A Dissertation

APPROVED FOR

THE DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

BY

Ralph A. Wheeler
Roger French
B. M. Fung
Gregory A. Parker

For my parents
Paul and Betty Grafton

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Abstract

Certain aspects of photosynthesis remain unexplained. The functions of the quinones present in both plant and bacterial photosynthetic reaction centers is still not completely understood. Presented here is a detailed computational study of a variety of properties of various quinone species important to photosynthetic processes. It is first demonstrated that the hybrid Hartree-Fock/density functional method Becke3LYP (B3LYP) can accurately predict the structure of many neutral methyl-substituted 1,4-benzoquinones and 1,4-naphthoquinones. Structures of many reduced and protonated quinones are also presented and discussed in detail. It is shown that qualitative spin densities and accurate isotropic hyperfine coupling constants may also be calculated for quinone radical anions, although the heavy atom hyperfine coupling constants show some basis set dependence. The B3LYP method is also demonstrated to reproduce the ordering of experimentally determined vibrational frequencies of quinones and a variety of their reduced and protonated forms in nearly all instances. Qualitative shifts in vibrational frequencies upon protonation or hydrogen bonding are predicted and discussed relative to experiment. In order to properly compare and analyze the vibrational spectra calculated for similar species of quinones, the vibrational projection analysis method is developed and presented as a simple but powerful technique that should be applicable to a wide range of systems. Next, it is shown that the B3LYP method can be used to predict adiabatic electron affinities for a variety of quinones to within 0.05 eV of experimental values. When this ability is combined with molecular dynamics free energy perturbation methods, accurate quinone aqueous one-electron reduction potentials may be obtained. This method generally reproduces reduction potentials to within 0.20 eV of experiment, although exceptional accuracy (within 0.05 eV) was obtained for ubiquinone and

plastoquinone. Lastly, molecular dynamics simulations of ubiquinone binding in the photosynthetic reaction center of *Rhodobacter sphaeroides* are presented which support the theory that the neutral and radical anion Q_B quinones bind in different locations. The differences in binding are attributed to differing protonation states of the nearby amino acids GLU L212 and ASP L213. It is suggested that the movement of the quinone between sites may depend on the protonation state of L213 particularly, and that L212 is likely to be predominantly protonated in all cases. The relevance of these results to the experimentally observed activation energy for electron transfer to Q_B is discussed with respect to previous theories.

CHAPTER 1

Introduction to Bacterial Photosynthesis and the Methods Used In This Computational Study^{1,2}

1.1 Bacterial Photosynthesis

Photosynthesis is the conversion of light energy into chemical energy. It is the primary source of energy for virtually all known living organisms. Despite its importance as a fundamental energy storage process, there are still unanswered questions about the exact reactions that take place inside plant photosynthetic reaction centers (RC). One way to study photosynthesis is by investigating the RC's of some photosynthetic bacteria, which provide excellent models of plant photosystems.^{3,4} Several atomic-scale structures of the bacterial RC's are available.^{5,6} The RC's are made up of three protein subunits, commonly designated as heavy (H), medium (M), and light (L). The chromophores are spatially organized inside the protein in a manner similar to that shown schematically in Figure 1-1. The entire quinone reduction cycle of bacterial photosynthesis is shown in Figure 1-2. Excitation of the special pair of bacteriochlorophylls causes electron transfer to the primary acceptor pheophytin (H_A), which is followed by electron transfer to the primary quinone (Q_A), and on to the secondary quinone (Q_B). The cycle repeats itself (with or without proton transfer to Q_B), with the net result of transferring two electrons and two protons to Q_B to form a quinol, which then leaves the reaction center to be replaced by a new quinone. The resulting quinol helps form a charge gradient which is used to drive

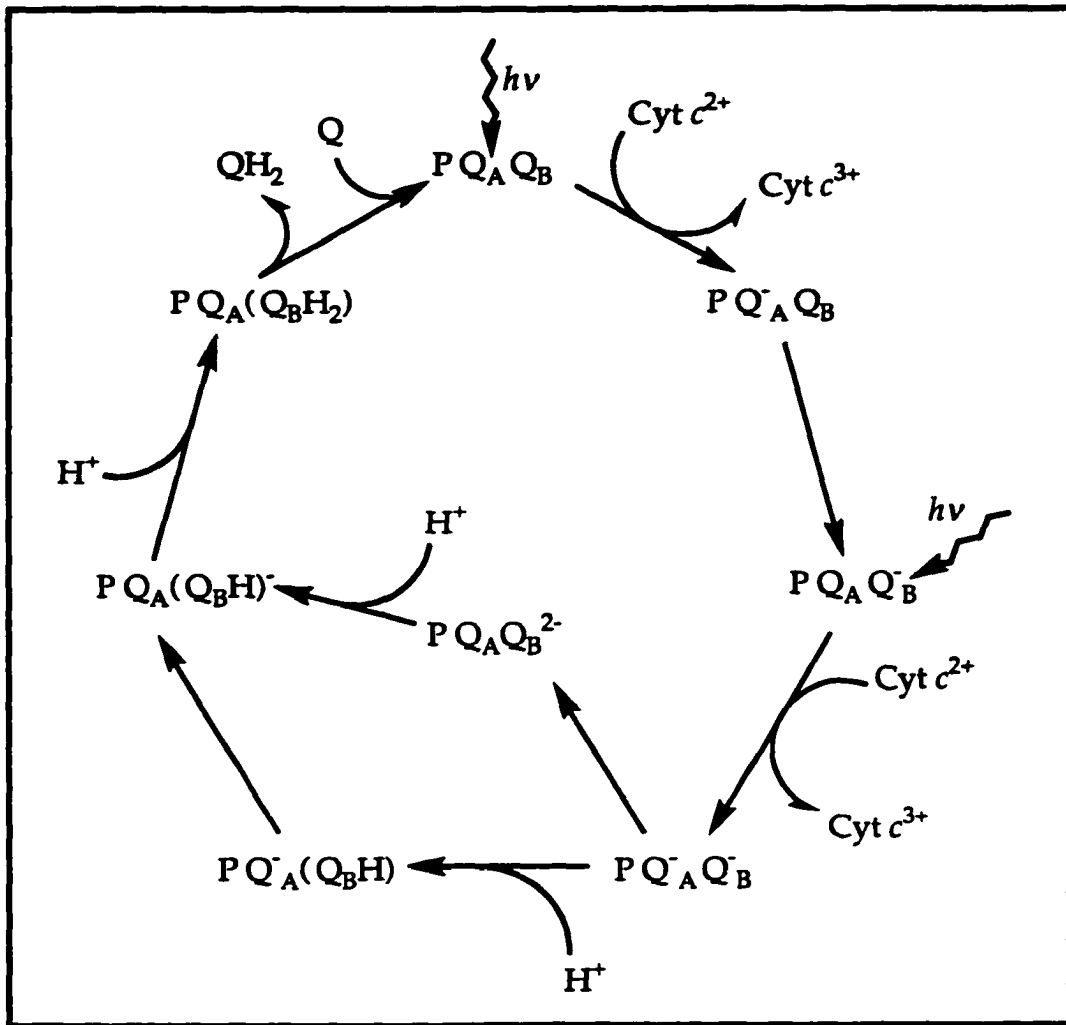


Figure 1-1. The Quinone Reduction Cycle in Bacterial Photosynthesis. Proton transfer to the secondary quinone may occur before or after the second electron is transferred. After the second protonation step, the quinol leaves the reaction center to be replaced by a new quinone.

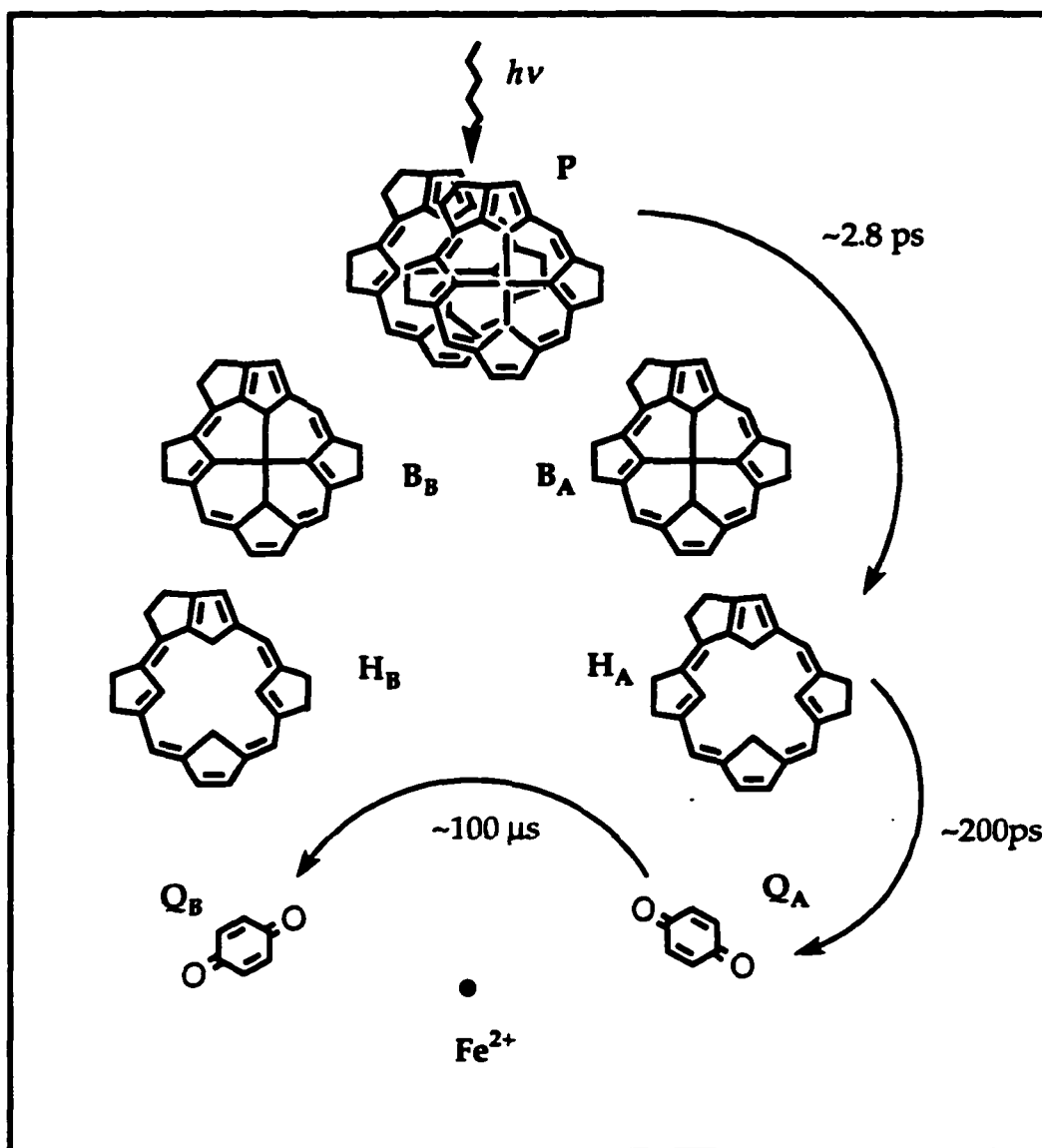


Figure 1-2. Approximate Arrangement of the Chromophores and Cofactors in Bacterial Photosynthetic Reaction Centers Along with Approximate Forward Electron Transfer Rates. P is the special pair of bacteriochlorophylls, B's are the accessory bacteriochlorophylls, H's are the bacteriopheophytins, and Q's are the primary and secondary quinones. Electron transfer occurs almost exclusively along the active, A branch.

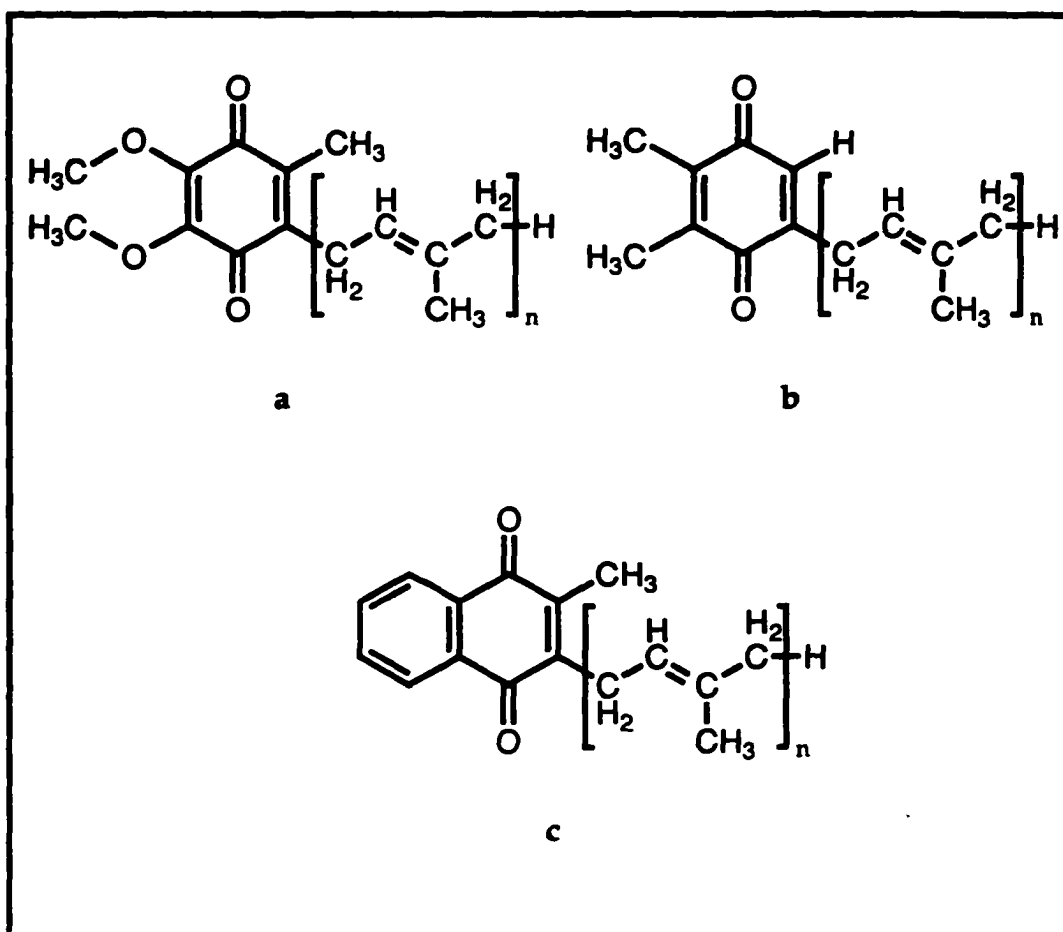


Figure 1-3. Biologically Important Quinone Species. Ubiquinone (a) is used as the primary (Q_A) and secondary (Q_B) quinones in the reaction center of *Rb. sphaeroides*, while in *Rp. viridis*, menaquinone (c) is used as the secondary electron acceptor. In plant photosystems, it is plastoquinone (b) which is used as the electron acceptor.

the synthesis of critical biological compounds such as the energy storage molecule ATP. The RC is nearly C_2 symmetrical along an axis through the special pair and non-heme iron, and this close symmetry is part of an as-yet unanswered question: why does electron transfer proceed preferentially down only one side of the reaction center (the active, A branch)?

One important factor which may affect the function of the quinones in the RC's is their binding sites. In the RC of the purple bacterium *Rhodobacter sphaeroides*, for example, the Q_A and Q_B sites are naturally occupied by the same quinone, ubiquinone-10 (see Figure 1-3), but the function of each quinone is quite different. Q_A is never protonated, and acts only as an electron shuttle to Q_B , which is more loosely bound and leaves the reaction center after it accepts two hydrogens. Experimental substitution of the native ubiquinone by other quinones has shown that the reduction potential of Q_B is not the determining factor in the rate-limiting step of electron transfer.⁸¹ Instead, the difference seems to be in the local binding environments of the two quinones. The binding site of Q_A has few protonatable protein residues nearby, while Q_B has several in its vicinity. The precise mechanisms for the coupling of proton uptake by the protein (and by Q_B) to electron transfer to Q_B are not known.⁷

Many experiments have been conducted to study the function of the quinone acceptors and the formation of quinone radical anions in bacterial RC's. A wide variety of quinones have been studied both *in vitro* and *in vivo* with techniques ranging from infrared spectroscopy to X-ray crystallography. More recently, the continued advances in computer power and computational methodologies have made it possible to conduct theoretical studies of quinones in many different environments. We present here the results of extensive

computational studies of various quinones in different reduced and protonated forms in the gas phase, aqueous solution, and in the *Rb. sphaeroides* RC protein environment. We show that many crucial properties of quinones important to photosynthesis can be accurately and reliably predicted. We provide extensive data that will help both to interpret the results of complex experiments and also to suggest new experiments and future theoretical studies.

1.2 Quantum Chemical Methods

1.2.1 Density Functional Theory

All density functional (DF) methods are based on the set of one-electron Kohn-Sham^{8,9} equations

$$\hat{H}_{KS}\psi_i(r_1) = \left[-\frac{1}{2}\nabla^2 + \sum_A \frac{-Z_A}{|R_A - r_1|} + \int \frac{\rho(r')}{|r_1 - r'|} dr' + V_{XC} \right] \psi_i(r_1) = \epsilon_i \psi_i(r_1) \quad (1.2-1)$$

where R_A and Z_A are the coordinates and charges of the N atomic nuclei, and ρ is the electron density. This is very similar to the formalism of Hartree-Fock (HF) theory,^{10,11} except for the inclusion of V_{XC} , a term which combines all non-classical interactions, including electron correlation and exchange. In principle, these equations are exact, except that the functional form of V_{XC} is not known. The simplest approximation of this non-classical term is the local density approximation, where the non-classical energy contributions are dependent only on the local charge density, such that

$$V_{XC}[\rho(r)] = \int \epsilon_{xc}(\rho(r))\rho(r)dr \quad (1.2-2)$$

where $\epsilon_{xc}(\rho)$ is the exchange-correlation energy of each particle in a uniform electron gas of density ρ . Other approximations are available,¹²⁻¹⁴ such as the generalized gradient approximations where V_{xc} depends on the gradient of the charge density. DF methods have the advantage of including the non-classical interactions at a very modest computational cost over simple HF theory.

In this study, we rely primarily on a method which combines DF and HF theory, the so-called hybrid HF/DF Becke3LYP (B3LYP) method. Hybrid HF/DF methods use a weighted sum of Slater's local exchange approximation,¹⁵ HF, Becke's gradient-corrected exchange functional,¹⁶ Lee, Yang, and Parr's gradient-corrected correlation functional,¹⁷ and the local correlation functional of Vosko, Wilk, and Nusair,¹⁸ so that the total exchange and correlation energy, E_{xc} , may be written as

$$E_{xc} = (1 - a_0)E_X^{Slater} + a_0E_X^{HF} + a_XE_X^{Becke} + a_cE_C^{LYP} + (1 - a_c)E_C^{VWN} \quad (1.2-3)$$

where the parameters a_0 ($= 0.20$), a_X ($= 0.72$), and a_c ($= 0.81$) were chosen to reproduce experimental heats of formation.¹⁹ Because of this empirical fitting, HF/DF methods are not strictly *ab initio*, but may be viewed as very advanced semi-empirical methods. Except where noted, all quantum calculations were performed with the B3LYP method as implemented in the Gaussian92/DFT²⁰ and Gaussian94²¹ quantum chemistry programs.

1.2.2 Basis Sets

A basis set is meant to provide an approximation to the atomic orbitals.^{22,23} In this study, we use Gaussian-type orbitals, whose functional form is

$$\phi_j = \sum_i C_{ij} \eta_i \quad (1.2-4)$$

where ϕ_j is an orbital made up of a linear combination of normalized Gaussian functions, η_i , whose cartesian representation is written as

$$\eta(\alpha) = N_\alpha x^k y^l z^m e^{-\alpha r^2} \quad (1.2-5)$$

where N_α is a normalization constant, and α is the effective nuclear charge. The exponents k , l , and m sum to give the orbital type (0 = s orbital, 1 = p orbital, etc.). In most quantum calculations presented here, the split-valence plus polarization 6-31G(d) basis set is used. It represents a reasonable compromise between basis set size and computational efficiency for systems of the size studied here. Other basis sets are used as noted for other calculations including accurate electron affinities and spin properties.

1.2.3 Spin Properties

All spin densities reported in this study were determined by Mulliken population analysis²⁴, and are therefore expected to be only qualitatively correct. Isotropic hyperfine coupling constants (hfcc's) were generally determined at both the B3LYP/6-31G(d) level and with the larger Chipman [632|41] basis set^{25,26} (which includes more diffuse and polarization functions on heavy atoms, as well

as a tighter, or smaller, s-function on hydrogens) in single-point calculations on B3LYP/6-31G(d) geometries. The Chipman basis set was designed specifically to provide reliable spin density properties. The hfcc's may be determined from the Fermi contact parameter,²⁷ or spin density at the nucleus, $\rho(N)$, (which is calculated directly in GAUSSIAN94) using the following formula,^{28,29}

$$a_0 = [(8\pi/3) \cdot g \cdot g_N \cdot \beta \cdot \beta_N] \cdot \rho(N) \quad (1.2-6)$$

where a_0 is the hfcc (in Gauss), g is electronic g factor, β is the electronic Bohr magneton, and g_N and β_N are the analogous values for nucleus N . The terms in brackets can be reduced to a single factor (in units of Gauss) for each type of nucleus: i.e. 1595 for ^1H , 401.0 for ^{13}C , and -216.2 for ^{17}O . Although there are still questions to be answered about the ability of density functional-based methods to reproduce properties based on electronic spin,³⁰⁻³² there is also evidence that the Kohn-Sham equations, which are the basis of density functional theory, are less sensitive than MO calculations to spin contamination³³ (which is the contamination of the wave function by higher spin states, causing it not to be an eigenfunction of $\hat{S}^2$). Hyperfine coupling constants may also provide a more stringent test of the computational methods used than spin densities alone since they are proportional to the spin density precisely at the nucleus.

1.3 Molecular Dynamics Methods

1.3.1 Molecular Dynamics Theory

All molecular dynamics (MD) calculations presented in this work were performed with the AMBER suite of computer programs.³⁴⁻³⁶ The basic principles of MD calculations have been thoroughly reviewed elsewhere.³⁷ In brief, MD simulations involve treating the system of interest as a group of point-mass atoms whose interactions are controlled by classical, Newtonian mechanics. Intramolecular pair potentials defined include harmonic potentials for bonds and angles and sinusoidal potentials for dihedral angles, where each individual parameter type is defined generally in terms of displacement from an equilibrium position. Atoms further than four bonds apart in any molecule or atoms in two different molecules interact through Lennard-Jones and Coulombic forces. So, the generic potential energy function V for a system of N atoms, each with some position $\mathbf{r}$, may be represented by

$$\begin{aligned} V(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N) = & \sum_{bonds} \frac{1}{2} K_b (b - b_0)^2 + \sum_{angles} \frac{1}{2} K_\theta (\theta - \theta_0)^2 \\ & + \sum_{dihedrals} K_\phi [1 + \cos(n\phi - \delta)] + \sum_{pairs(i,j)} [A_{12}(i,j) / r_{ij}^{12} - B_6(i,j) / r_{ij}^6] \\ & + q_i q_j / (4\pi\epsilon_0 r_{ij}) \end{aligned} \quad (1.3-1)$$

where K 's represent force constants for particular parameters, and variables which are subscripted zero define the equilibrium values. For dihedrals, δ represents the current position, and n and ϕ are the multiplicity and phase, respectively, of the cosine function which defines a particular dihedral potential. A_{12} and B_6 are parameters which define the Lennard-Jones interactions of a particular atom type.

The variables q_i and q_j are the partial charges assigned to the atoms in a pair, and r_{ij} is the distance between the atoms. In some cases, another interaction is defined

$$\sum_{pairs(i,j)} [C_{12}(i,j) / r_{ij}^{12} - D_{10}(i,j) / r_{ij}^{10}] \quad (1.3-2)$$

to specifically mimic the “hydrogen bond” energy, with C_{12} and D_{10} as defined parameters for the interaction. The subsequent trajectories of the atoms in the simulated system are given by Newton’s equations of motion:

$$d^2 \mathbf{r}_i(t) / dt^2 = \mathbf{F}_i / m_i \quad (1.3-3)$$

$$\mathbf{F}_i = -\partial V(\mathbf{r}_1, \mathbf{r}_2 \dots \mathbf{r}_N) / \partial \mathbf{r}_i \quad (1.3-4)$$

The time steps over which these equations are solved are typically around 1 to 2 fs, which is allowed by holding bond lengths constant using the SHAKE algorithm,^{38,39} thus removing bond vibrations. The temperature of the system may be maintained at a desired value by coupling the system to a heat bath using a technique such as the Berendsen coupling algorithm.⁴⁰ Unless otherwise noted, all MD simulations presented in this work were performed at 300±20K using SHAKE with a time step of 1 fs.

Two issues arise in regards to the system size which should be addressed. The first is how to simulate a bulk system with only a few hundred molecules, and the second is how to limit the number of non-bonded interactions must be calculated. The most common way of simulating a near-infinite system with a finite number of atoms is to apply periodic boundary conditions. Periodic

boundaries allow molecules on the right side of the system to “see” molecules on the left side, those on top to “see” those on bottom, etc. Applied in this manner, periodic boundary conditions eliminate edges, or discontinuities, in the system, thus simulating an “infinite” box. Unless otherwise noted, all solution-phase systems presented in this work use periodic boundary conditions and constant pressure⁴¹ (1 atm), while gas-phase and protein simulations do not.

The second problem that must be addressed involves computational tractability. If periodic boundary conditions are applied, then there is a very large number of possible non-bonded interaction pairs to include in the calculation. Several methods have been developed to reduce the number of non-bonded interactions which must be evaluated.⁴²⁻⁵⁰ The simplest method which may be used is a non-bonded interaction cutoff, which means that all interactions beyond a certain distance from any one atom are ignored. This technique is used with a 10 Å cutoff in the reduction potential calculations described in Chapter 4, and with a 15 Å cutoff in the protein simulations described in Chapter 5. It should be noted that cutting off distant interactions in this manner affects free energy calculations in a quantifiable way (see Sections 1.3.3 and 4.1).

1.3.2 Molecular Force Fields

As mentioned in Section 1.3.1, MD calculations require that various parameters (bond lengths, force constants, partial atomic charges, etc.) for the simulated molecules be known. This set of parameters, which completely describe a system and its interactions, is known as a force field. We used the TIP3P force field⁵¹ to describe water molecules throughout this study, and, unless otherwise noted, we used the Weiner et al. united-atom AMBER force field⁵² to describe

proteins. Force fields for 1,4-parabenzquinone and several halogen-substituted derivatives have been developed previously,^{53,54} but for the quinone molecules and their radical anions examined in this study, no force fields were available. For these molecules (and in some other cases as mentioned) force fields were derived from quantum mechanics calculations (see Section 1.2) as a part of this research. Equilibrium bond distances, bond angles, and dihedrals were taken from fully optimized geometries. Force constants were obtained by taking the diagonal elements from the cartesian force constant matrix which had been transformed to a set of internal coordinates chosen according to the scheme of Boatz and Gordon⁵⁵ using the GAMESS quantum chemistry program.⁵⁶ Partial atomic charges, unless otherwise noted, were obtained by fitting to the calculated electrostatic potentials using the CHelpG charge fitting algorithm.^{57,58} Lennard-Jones parameters were taken from similar atom types defined in the Weiner et al. force field.

1.3.3 Free Energy Calculations

It is possible to determine accurate solvation free energy differences ($\Delta\Delta G$) using MD simulations.⁵⁹⁻⁶² Because it is unreliable to attempt to obtain small free energy differences by simply subtracting one large number from another, an alternative method was developed⁶³ which involves perturbing one system into another in small steps by creating a weighted Hamiltonian

$$H(\lambda) = \lambda H_B + (1 - \lambda) H_A \quad (1.3-4)$$

such that H_A and H_B define the two systems of interest, and λ is a parameter which varies from 0 to 1, thus effectively perturbing system A into system B. One can then calculate the free energy difference between systems A and B by

$$G_B - G_A = \Delta G_{AB} = \sum_{\lambda=0}^1 -RT \ln \langle e^{-\Delta H'/RT} \rangle_{\lambda} \quad (1.3-5)$$

where $\langle \rangle_{\lambda}$ denotes an ensemble average over a system defined by H_{λ} . This method is known as free energy perturbation, and is used for the reduction potential calculations presented in Chapter 4 with λ varying in 0.05 increments, meaning a total of 21 steps is required to fully perturb one system to another. At each value of λ , the system is first equilibrated, then data is gathered for the ensemble average. The total number of time steps used for equilibration and data gathering controls the total length of the simulation.

When calculating free energy differences in a simulation in which a non-bonded cutoff has been used (see Section 1.3.1) and total charge on the system is being perturbed, it is necessary to account for the difference in interaction energies of species with the bulk solvent outside the cutoff. This is the Born charging correction,⁶⁴⁻⁶⁶ and its application is discussed more fully in Section 4.1.

1.4 Vibrational Projection Analysis of Normal Modes

1.4.1 Introduction

Advances in computational chemistry over the past 20 years have made the calculation of vibrational frequencies and vibrational modes of small molecules routine and promise access to the same data for increasingly large and complicated structures. Analytical gradient methods⁶⁷ are now incorporated in most quantum chemistry programs and allow the accurate determination of minimum energy geometries as well as vibrational frequencies and modes for many molecules. Computer programs are also becoming available to calculate normal vibrational modes of proteins based on molecular mechanics calculations.^{35,68} These stunning advances in computational technology have been accompanied by equally remarkable progress in spectroscopic methods. Collecting vibrational spectra for small molecules is now routine and advances at the forefront of vibrational spectroscopy^{69,70} make it possible to determine vibrational frequencies for large, complex structures such as proteins and polymers.^{71,72} Vibrational difference spectroscopy, for example, now provides the means to compare experimental spectra for oxidized and reduced protein side-chains directly.⁷³ The easy access to and convenience of quantum chemical, molecular mechanics, and molecular dynamics computer programs, coupled with the amazing progress in computational and experimental methods, means that calculated vibrational frequencies and modes are now an important complement to experimental spectral measurements. It also means data can be computed rapidly and in great quantities, even for very large systems. For example, even a relatively small calculation for the simple, highly symmetrical benzene molecule can produce nearly two million

bytes of data. The proper analysis of and convenient comparisons among such copious data can be both difficult and time consuming. This section describes original work to develop a new method which relies on vector projections for quickly and quantitatively analyzing and comparing computed vibrational modes. The vibrational projection analysis method described here is also compared to complementary methods for comparing vibrational modes, such as total energy decompositions (TED)⁷⁴ and graphical visualization.

Vibrational spectra can be used to infer such things as changes in bonding, electronic state, or local environment, but drawing these inferences from experimental or theoretical data is often a complicated matter. Frequently, it is necessary to compare the spectra of two systems and note changes in the vibrational frequencies. However, mode mixing and related phenomena induced by a perturbation can make this process difficult, especially if the initial mode assignments are uncertain. Even the computational chemist, with pictorial and animated normal modes of vibration available, can find that confidently matching the modes of similar systems is tedious at best, and can become quite difficult if the two systems differ in such things as chemical substituents or oxidation states, which can dramatically affect the descriptions of vibrational modes.

TED is the numerical method currently used most often by computational chemists to help match vibrational modes between different molecules.⁷⁵ The TED was originally developed to provide a quantitative breakdown of the energetic contributions of a molecule's internal coordinates to each of the vibrational modes. Using this method, one could describe a vibration of a molecule such as benzene as 95% C-H stretch and 5% C-C stretch. TED calculation has been incorporated in commonly used quantum chemistry programs such as

GAMESS, and is essentially the only quantitative tool available to describe normal modes.

If used for its intended purpose—describing individual normal modes of a single system—then TED analysis can be quite useful. However, computational chemists have come to rely on TED's to assist in the comparison of vibrational modes from different systems, and in this regard, TED analysis has some important shortcomings. First, and least seriously, the results of the analysis can depend on the user's choice of internal coordinates. Methods have been proposed for selecting internal coordinates,⁵⁵ and our own experience shows that the effect of choosing different sets is primarily expressed in the low frequency part of the vibrational spectrum. Nonetheless, internal coordinates must be selected with care to assure reliable results. Secondly, comparing the TED's from two molecules does not strictly result in a quantitative comparison. In the case of the quinones studied here, for instance, there are vibrational modes in both the neutral and the radical anion species which involve strong mixing between the C=C and C=O stretches, with some of these modes essentially 1:1 in energetic contribution. A comparison of the TED analysis in these cases is uninformative because of its most serious shortcoming: information about the phase of the atomic displacements is not taken into account, and only a qualitative visual analysis provides a final determination.

Because of the importance of comparing vibrational normal modes, and because of the limitations of TED analysis for this purpose, we have developed a simple but effective technique for giving fast, quantitative comparisons between the calculated normal modes of similar systems that takes into account the vibrational phases and does not require the user to select a set of internal

coordinates. Our method, ViPA (for Vibrational Projection Aalysis), treats normal modes as simple vectors which define an orthonormal vector space, and projects the modes of another molecule onto this vector space to make the quantitative comparison. This procedure generalizes the work of Duschinsky^{76,77} by including comparisons of molecules with different numbers of atoms.

We have made available a simple software routine for performing vibrational projection analysis,¹ and as an example of the power of ViPA, we have shown how it can be used to facilitate the quantitative comparison of the normal modes of benzene, phenol, phenol-*d*5, and phenol radical cation.² We demonstrate that ViPA is superior to TED analysis for mode comparisons. Vibrational analysis using ViPA need not be limited to cases of perturbations by chemical substitution, isotopic masses, and different oxidation states, as in the examples we present here. The technique could also be used to compare modes calculated with different methods and/or basis sets and even to compare modes from molecules in different electronic states, which may make it possible to estimate Franck-Condon factors, which are related to the similarity of vibrational wave functions of the ground and excited states of a molecule.⁷⁷ Additionally, normal modes may be calculated for systems perturbed by non-covalent contacts—including individual molecules, proteins, polymers, hydrogen-bonded systems (such as those described in Chapter 3) and a variety of solid-state systems—so we anticipate that ViPA may become a useful tool for analyzing vibrational modes of many different structural types.

1.4.2 Mathematical Theory Behind ViPA

The classical theory of vibrations for polyatomic molecules⁷⁸ demonstrates that an N-atom molecule has 3N degrees of freedom, where 6 degrees of freedom account for the rotation and translation of a non-linear molecule (5 in the case of a linear molecule), and the remaining 3N-6 describe internal vibrations. Each degree of freedom is associated with a single atomic displacement. If the displacements are given in terms of mass-weighted cartesian displacements, q_i , where

$$q_i = m_i^{1/2}(x_i^o - a_i) \quad (1.4-1)$$

x_i^o is the equilibrium position, a_i is the amount of displacement, and m_i is the mass of the atom, then the normal modes form an orthonormal basis set which spans the space of possible nuclear motions. (Throughout this discussion, elements of matrices are represented by lower case subscripted variables, while vectors and matrices themselves are represented by uppercase variables with single or double underlining, respectively.) The 3Nx3N symmetric force constant matrix $\underline{\underline{F}}$ is easily constructed using analytical gradient techniques in quantum chemistry programs such as Gaussian94,²¹ and we do not reiterate it here.

Once $\underline{\underline{F}}$ is obtained, diagonalization routines such as the Jacobi or Givens-Householder methods^{79,80} may be applied to determine the set of eigenvectors (normal modes), $\underline{\underline{Q}}$, and their corresponding eigenvalues (which are proportional to the vibrational frequencies), λ_{3N} . Each eigenvector, $\underline{\underline{Q}}_j$, is a 3Nx1 column vector which defines a single normal mode j . As mentioned earlier, if we use

mass-weighted cartesian displacement coordinates, the resulting eigenvectors are normalized and orthogonal, i.e.

$$\underline{Q}_j^T \underline{Q}_k = \delta_{jk} \quad \begin{array}{l} \delta_{jk} = 1 \text{ if } j = k \text{ and} \\ \delta_{jk} = 0 \text{ if } j \neq k \end{array} \quad (1.4-2)$$

The set of vectors containing the mass-weighted atomic displacements corresponding to each normal mode then define a 3N-dimensional orthonormal vector space, and any arbitrary vector $\underline{P}$ can be projected onto this vector space as:

$$\underline{P}_Q = \sum_{j=1}^{3N} p_j \underline{Q}_j \quad (1.4-3)$$

where the coefficients p_j are defined by

$$p_j = \underline{P}^T \underline{Q}_j \quad (1.4-4)$$

and the subscript Q in equation 1.4-3 denotes that this is a projection of $\underline{P}$ on $\underline{Q}$, and not necessarily equal to $\underline{P}$ itself. The length, or norm, of the projection of $\underline{P}$ is given by the following equation:

$$\|\underline{P}\|_Q^2 = \sum_{j=1}^{3N} p_j^2 \quad (1.4-5)$$

For the present application, we will not use just an arbitrary vector $\underline{P}$, but will instead use a normal mode from a second molecule with N' atoms, with its own set of normal modes denoted here as $\underline{Q}'$. The assumption is made that for each atom in the basis molecule, there is a corresponding atom in roughly the same location relative to the other atoms in the second molecule (meaning that the molecules must be oriented similarly with respect to the coordinate axes used), and that the atomic displacement coordinates of the first N atoms of both molecules are ordered similarly before constructing $\underline{F}'$. It is not necessary that $N = N'$, only that $N \leq N'$. A complete projection of each mode $\underline{Q}'_k$ onto the vectors $\underline{Q}_j$ results in a $3N \times 3N'$ matrix, which we denote here as $\underline{C}$, whose elements c_{kj} would be defined by

$$c_{kj} = \underline{Q}'_k{}^T \underline{Q}_j \quad (1.4-6)$$

Since the individual normal modes $\underline{Q}'_k$ are normalized by the condition described in equation 1.4-2, equation 1.4-5 shows that, usually,

$$\|\underline{Q}'_k\|_Q^2 = \sum_{j=1}^{3N} c_{kj}^2 \equiv 1 \quad (1.4-7)$$

and the percentage contribution of each normal mode j of the basis molecule to each normal mode k of the second molecule is simply

$$c_{kj}^{\%} = 100c_{kj}^2 \quad (1.4-8)$$

Since the second molecule may have a greater number of atoms than the basis molecule, or may differ in some other way, there could be some displacements which are not describable in the basis defined by the first molecule. For each normal mode k in the second molecule, we define the d-factor, $d_{kj}^{\%}$, as the percent which is not represented by the basis molecule by

$$d_{kj}^{\%} = 1 - c_{kj}^{\%} \quad (1.4-9)$$

Vector projection analysis results are not difficult to interpret: any mode of the second molecule may be described as a linear combination of modes from the basis molecule, with the d -factor, $d_{kj}^{\%}$, representing the percentage not described by the basis molecule. This procedure essentially automates the process of matching modes between similar molecules, as one may determine a match by looking for the basis mode which contributes the highest percentage to the mode of interest in the second molecule.

Vibrational projection analysis was performed using the ViPA program which has been described and made available elsewhere.¹ It should be noted that the ViPA algorithm uses only the force constant matrix from the quantum calculations and determines normal modes internally, primarily because the modes output by Gaussian94 are not computed using the mass-weighted cartesian displacement coordinates, and are therefore not orthogonal, which is a property important to the function of the ViPA method (see equation 1.4-2 and the subsequent discussion). Total energy distributions presented in this work were

obtained using the GAMESS quantum chemistry program with the force constant matrix generated by Gaussian94. Internal coordinate sets for total energy distributions were chosen using the procedure described by Boatz and Gordon.⁵⁵

1.4.3 Deuterated Water: A Brief Example

As a simple example system, we compare the vibrational modes of H₂O to those of its deuterated analog, D₂O. The force constant matrix for this example was obtained from Gaussian94 after running a geometry optimization and frequency calculation on H₂O, using the HF/6-31G(d) method. Sample output from the ViPA method is reproduced below.

H ₂ O Modes (Frequencies) (1336) (2936) (3071)	Modes of D ₂ O as % of H ₂ O								
	1 (-30)	2 (-30)	3 (-30)	4 (-1)	5 (0)	6 (0)	7	8	9
1 (-43)	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2 (-42)	0.0	99.4	0.0	0.5	0.0	0.0	0.0	0.0	0.0
3 (-40)	0.0	0.0	98.5	0.0	0.0	1.5	0.0	0.0	0.0
4 (-1)	0.0	0.0	0.0	0.0	98.4	0.0	0.9	0.5	0.1
5 (0)	0.0	0.5	0.0	98.4	0.0	0.0	0.1	0.0	1.0
6 (0)	0.0	0.0	1.5	0.0	0.0	98.5	0.0	0.0	0.0
7 (1826)	0.0	0.0	0.0	0.1	0.9	0.0	98.9	0.1	0.0
8 (4072)	0.0	0.0	0.0	0.0	0.5	0.0	0.2	99.3	0.0
9 (4190)	0.0	0.1	0.0	0.9	0.1	0.0	0.0	0.0	98.9
d-factor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

From the output, it is clear that the mode 7 of D₂O matches mode 7 of H₂O with a similarity of 98.9%, and includes very small contributions from other modes. Similar results are obtained for the last two vibrational modes. The rotational and translation “modes” (modes 1 through 6 in each molecule) also match very closely, except that translational modes 4 and 5 have been reversed in

D₂O relative to H₂O. The *d*-factors are consistently 0.0 for all modes, indicating the H₂O normal modes provide an excellent basis set for the normal modes of D₂O. Applications to more complex systems are submitted for publication and are described in Chapters 2 and 3.

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CHAPTER 2

A Comparison of the Properties of Various Fused-Ring Quinones and Their Radical Anions using Hartree-Fock and Hybrid Hartree-Fock/Density Functional Methods^{1,2}

2.1 Introduction

2.1.1 Background and the work of others

The quinones and their one-electron reduced forms, or semiquinone radical anions, occupy a central place in electron transfer chemistry and biological energy conversion.³⁻⁶ For example, quinones are known to play a crucial role in oxido reductases⁷⁻¹⁴ and semiquinones are known intermediates in the actions of some anti-tumor drugs¹⁵. In both plant and bacterial photosynthetic reaction centers, quinones act as primary and secondary electron acceptors,¹⁶⁻¹⁹ and evidence exists that semiquinone radical anions are formed and remain stable for reasonably long times in such systems.⁶ It has long been known that the blood clotting cascade involves a series of proteins, such as prothrombin, which cannot be produced in a mature form if there is a deficiency of vitamin K, a 1,4-naphthoquinone derivative.^{20,21} Other studies have identified vitamin K-dependent proteins which seem to be involved in calcium homeostasis.²¹⁻²³ Vitamin K is found naturally in two forms. The K₁ form, or phylloquinone (Figure 2-1a), is manufactured by plants and consists of a 2-methyl-1,4-naphthoquinone “head” structure with a carbon chain “tail” substituent at the 3- position made up of a prenyl unit followed by n saturated 5-carbon units. By far the most common form of K₁ has $n=3$, and

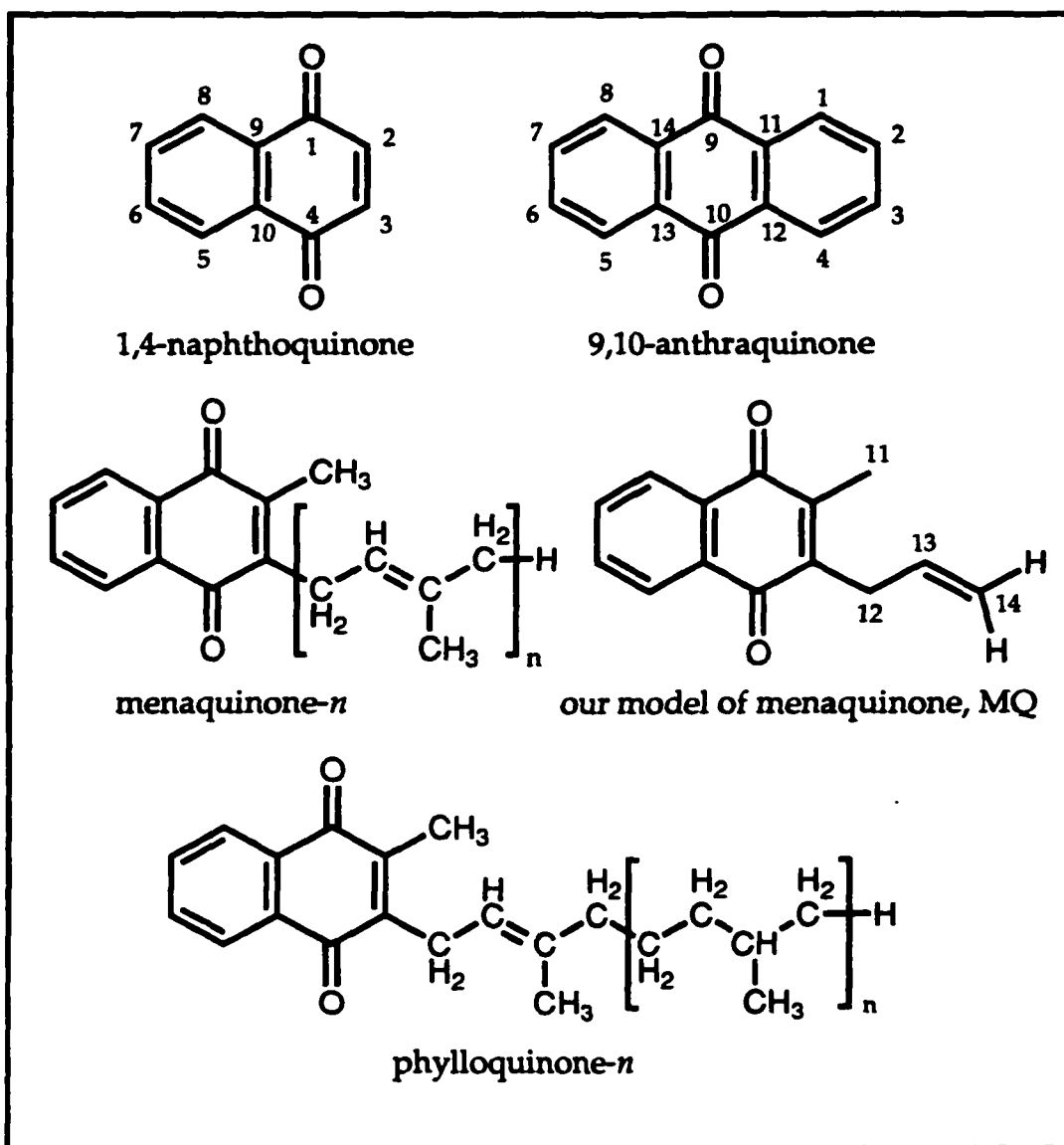


Figure 2-1. The Fused-Ring *para*-Quinones and Numbering Schemes Used in This Work.

the general term “phylloquinone” is usually used to name this species in particular. The other naturally occurring form of vitamin K, K₂, is menaquinone (Figure 3-1b). Menaquinones have the same head group as the phylloquinones, but a different chain substituted at the 3- position. This *n*-unit chain is the distinguishing feature of specific menaquinones, which are usually identified as menaquinone-*n*. Both K₁ and K₂ are involved in the molecular biology of a variety of vitamin K dependent proteins.^{24,25} Vitamin K₂ is synthesized by bacteria,²⁶⁻²⁸ where it serves as an acceptor in many different electron transport systems.⁴ For instance, *Escherichia coli* (*E. coli*) has respiration pathways which are dependent on vitamin K to mediate electron transport.^{26,29-32} Photosystem I¹⁶⁻¹⁸ and the bacterial photosynthetic reaction center of *Rhodospseudomonas viridis*¹⁹ each use forms of vitamin K as electron acceptors. Simpler, but similar, compounds have been used as models of menaquinone in various theoretical and experimental studies to determine the function of the native quinone.³³⁻³⁸ Some of these compounds include the parent 1,4-naphthoquinone (NQ), 2-methyl-1,4-naphthoquinone (2NQ, also known as vitamin K₃), and 2,3-dimethyl-1,4-naphthoquinone (23NQ). Various properties of these molecules, such as their fundamental vibrational frequencies³⁹⁻⁴⁷ and their ESR hyperfine interactions,⁴⁸⁻⁵³ are used to infer binding sites and cofactor-protein interactions in the photosynthetic reaction center. A knowledge of the properties of the isolated molecules and their radical anions is therefore crucial for the interpretation of experimental results.⁵⁴

The molecular structures of the larger, more biologically important quinones such as menaquinone (MQ), plastoquinone (PQ) and ubiquinone (UQ) (see Figure 1-3) and their radical anions have received theoretical attention only

recently,⁵⁵⁻⁵⁸ and no experimental structures of the isolated molecules are currently available. However, low-resolution X-ray structures of the photosynthetic reaction centers of certain purple bacteria contain these molecules in their native protein environment,¹⁹ and a recent study examined the conformation of the isoprenyl side-chains.⁴⁹ There are experimental structures of some related quinones which may serve as models of the larger species. X-ray structures of 2-methyl-1,4-benzoquinone,⁵⁹ 2,3-dimethyl-PBQ,⁶⁰ 2,5-dimethyl-PBQ,⁶¹ 2,6-dimethyl-PBQ,⁶² 2,3,5,6-tetramethyl-PBQ⁶³ (duroquinone, DQ), 2-methyl-5,6-methoxy-PBQ⁶⁴ (UQ0), 1,4-naphthoquinone⁶⁵ (NQ), 2,3-dimethyl-NQ⁶⁶ (23NQ), and 9,10-anthraquinone^{67,68} (AQ) have been determined over the last fifty years. More recently, the gas-phase electron diffraction structures of PBQ⁶⁹, DQ⁷⁰, and AQ⁷¹ were reported.

Considering the wide-ranging importance of quinones as biological electron acceptors, it is somewhat surprising that the radical anions of the quinones have only recently received much attention. Of the semiquinone radical anions, only the simplest species, PBQ^{•-}, has been studied extensively with theoretical methods. More information on the other semiquinone anions has recently begun to appear, but in some cases, such as vitamin K and its analogues NQ^{•-}, 2NQ^{•-}, and 23NQ^{•-}, a detailed comparison of their properties has yet to be performed.

Although the vibrational spectra of NQ and AQ have been studied relatively thoroughly both by experimental⁷²⁻⁸³ and theoretical methods,⁸⁴⁻⁸⁷ there is very little spectral data in the current literature for the radical anions of these molecules. Similarly, the vibrational spectra of 2NQ and 23NQ, both idealized model compounds for vitamin K, have been studied,^{74,77,86,88,89} but a comprehensive analysis of the fundamental vibrational frequencies of these

molecules and their radical anions has not yet been conducted. The vibrational spectra of the most fundamental of the fused-ring *para* quinones, 1,4-naphthoquinone, has been investigated by others, and some early work concluded that NQ exhibits only one C=O stretching frequency.⁹⁰ More recent theoretical studies indicate that there are actually two distinct fundamental C=O stretching vibrational frequencies of NQ.⁸⁵

The ability to predict other properties of quinones accurately, such as the electron affinities,⁹¹ aqueous one-electron reduction potentials,⁹² and isotropic hyperfine coupling constants (hfcc's),^{55-58,93,94} has been reported only recently. The density functional and hybrid Hartree-Fock/density functional (HF/DF) methods have proven very useful in this area, and have been shown to give reasonable hfcc's for other organic π -radicals as well.^{56,95-101} In this study, we present a comparison of the geometries and harmonic vibrational frequencies of NQ, 2NQ, 2,3NQ, MQ, AQ and their associated radical anions, as well as the spin densities and isotropic hyperfine coupling constants of the radical anions. We compare and contrast these properties and discuss their importance in the interpretation of experimental results, including the question of Fermi resonance involving the C=O stretches of NQ. A detailed description of our methods and analysis techniques can be found in the Section 1.2.

2.1.2 Models of vitamin K

The model of vitamin K we used (MQ, Figure 2-1) is actually a simplified form of menaquinone-1 with the chain-terminal methyl groups replaced by hydrogens. Because the ring structure and first unit of the substituent chain in the K₁ and K₂ forms are identical, the calculations presented in this work are actually

representative of both the types of vitamin K. The optimizations of both the neutral and radical anion forms of MQ were begun with the C12-C13 bond nearly perpendicular to the ring, and a C2-C12-C13-C14 dihedral of approximately 240°, both of these orientations are consistent with the conformation found for menaquinone in the X-ray diffraction structure of *Rp. viridis*.¹⁰² Earlier calculations on the analogous molecules ubiquinone^{55,58} and plastoquinone⁵⁷ carefully searched the conformational space of the C2-C12-C13-C14 dihedral, and also found this chain conformation to be the lowest in energy. The optimizations were begun with a planar head group, but the geometry of the entire system was allowed to optimize freely.

2.2 Results and Discussion

2.2.1 Geometries of the simple fused-ring quinones and their radical anions

Table 2-1 compares the available experimentally determined geometries of NQ, 2-methyl-NQ (2NQ), and 2,3-dimethyl-NQ (23NQ) with the predictions of *ab initio* and density functional theories. When compared to the solid-state X-ray diffraction structure, the RHF/6-31G method⁸⁶ gives the best average reproduction of bond distances in NQ, with the hybrid B3LYP/6-31G(d) method slightly better than the gradient-corrected pure density functional BP86/6-31G(d) method,⁸⁵ which are both slightly worse than the UHF/6-31G(d) method. It is worth noting that the X-ray structure is distinctly asymmetric, implying that the ubiquitous crystal packing forces may have significantly altered the structure from that of the gas phase. Unfortunately, the gas phase structure of NQ, which would be the best basis of comparison for the methods, has not been determined. The UHF/6-

Table 2-1. Calculated Bond Distances (Å) and Bond Angles (deg) of 1,4-Naphthoquinone (NQ), 2-Methyl-1,4-Naphthoquinone (2NQ), and 2,3-Dimethyl-1,4-Naphthoquinone (23NQ).

	NQ				2NQ		23NQ	
	Expt. ⁶⁵ (X-ray)	RHF ⁸⁶	BP86 ⁸⁵	B3LYP	RHF ⁸⁶	B3LYP	Expt. ⁶⁶ (X-ray)	B3LYP
C1=O ^a	1.22	1.222	1.239	1.226	1.223	1.226	1.24	1.227
C4=O	1.21	1.222	1.239	1.226	1.224	1.227	1.23	1.227
C2-C3	1.31	1.327	1.356	1.344	1.332	1.350	1.34	1.358
C9-C10	1.39	1.397	1.418	1.409	1.395	1.407	1.40	1.404
C6-C7	1.37	1.388	1.408	1.399	1.389	1.399	1.37	1.399
C1-C2	1.48	1.477	1.488	1.485	1.490	1.501	1.47	1.495
C3-C4	1.45	1.477	1.488	1.485	1.473	1.478	1.45	1.495
C1-C9	1.43	1.483	1.496	1.492	1.483	1.491	1.45	1.489
C4-C10	1.46	1.483	1.496	1.492	1.482	1.490	1.49	1.489
C8-C9	1.39	1.388	1.406	1.398	1.388	1.399	1.40	1.398
C5-C10	1.36	1.388	1.406	1.398	1.388	1.397	1.39	1.398
C5-C6	1.43	1.386	1.401	1.393	1.386	1.394	1.39	1.393
C7-C8	1.41	1.386	1.401	1.393	1.386	1.393	1.39	1.393
Avg. Deviation from Expt.	-	0.020	0.030	0.027	-	-	-	0.015
O-C1-C2	118.5	-	-	120.5	-	120.6	119.0	120.8
O-C4-C3	118	-	-	120.5	-	120.6	120.0	120.8
C2-C1-C9	121.5	-	117.2	117.3	-	118.2	121.5	118.4
C3-C4-C10	123	-	117.2	117.3	-	117.4	120.8	118.4
C1-C2-C3	120.5	-	122.2	122.2	-	119.6	119.7	121.2
C4-C3-C2	117.5	-	122.2	122.2	-	124.0	120.7	121.2
C1-C9-C8	123	-	119.6	119.6	-	119.4	121.0	119.7
C4-C10-C5	123.5	-	119.6	119.6	-	119.9	120.2	119.7
C1-C9-C10	118	-	120.6	120.6	-	120.8	119.5	120.3
C4-C10-C9	117.5	-	120.6	120.6	-	120.0	117.7	120.3
C7-C8-C9	121	-	120.0	120.0	-	120.0	117.5	119.9
C6-C5-C10	121.5	-	120.0	120.0	-	119.9	118.2	119.9
C5-C6-C7	118.5	-	120.2	120.2	-	120.1	119.7	120.2
C8-C7-C6	119	-	120.2	120.2	-	120.2	123.2	120.2
Avg. Deviation from Expt.	-	-	2.9	2.9	-	-	-	1.6

^a Atom numbering is consistent with Figure 2-1.

31G(d) method does slightly better in reproducing X-ray bond distances in 23NQ, but not as well as B3LYP/6-31G(d), which has an average absolute deviation in bond distance of 0.015Å. We are aware of no experimentally determined structure of 2NQ for comparison.

When comparing the structures of the quinones listed in Table 2-1 to each other, some trends are identifiable in both experiment and theory. All bonds except those directly involved with the 2- or 3- position methyl-substitution remain virtually constant throughout the series, including the characteristic C=O and C2=C3 bonds. Bond angles also show little change across the series, except around the substituted positions. The fused ring maintains its benzene-like structure, even including the shared C9-C10 bond, with average bond lengths just under 1.40Å. When comparing these structures to the structure of MQ (determined with the B3LYP/6-31G(d) method and presented in Section 2.2.2), it may be seen that the structure of the ring system of 23NQ is almost identical to that of MQ, having bond distances usually within 0.003Å, which implies that the isoprenoid chain has the same effect on the local structure of the ring system as a methyl group.

Table 2-2 presents the calculated structures of the radical anions NQ^{•-}, 2NQ^{•-}, and 23NQ^{•-} as determined by the UHF/6-31G⁸⁶ and UHF/6-31G(d) molecular orbital based methods, as well as by the HF/DF B3LYP/6-31G(d) method. We are aware of no experimental determinations of the structures of these radical anions. Each method predicts a slight lengthening of the C2=C3 bond with further methyl substitution, as well as a small increase in the C-C bonds neighboring the substituted position. The C=O bond lengths remain virtually unchanged through the series for a particular method, but the UHF/6-31G method

Table 2-2. Calculated Bond Distances (Å) and Bond Angles (deg) of the Radical Anions of 1,4-Naphthoquinone (NQ^{•-}), 2-Methyl-1,4-Naphthoquinone (2NQ^{•-}), and 2,3-Dimethyl-1,4-Naphthoquinone (23NQ^{•-}).

	NQ ^{•-}			2NQ ^{•-}			23NQ ^{•-}	
	UHF ⁸⁶ 6-31G	UHF 6-31G(d)	B3LYP 6-31G(d)	UHF ⁸⁶ 6-31G	UHF 6-31G(d)	B3LYP 6-31G(d)	UHF 6-31G(d)	B3LYP 6-31G(d)
C1=O ^a	1.276	1.241	1.263	1.278	1.244	1.266	1.242	1.265
C4=O	1.276	1.241	1.263	1.275	1.240	1.263	1.242	1.265
C2-C3	1.371	1.372	1.381	1.373	1.375	1.385	1.381	1.392
C9-C10	1.406	1.402	1.423	1.405	1.400	1.421	1.397	1.420
C6-C7	1.405	1.404	1.410	1.406	1.404	1.410	1.405	1.411
C1-C2	1.418	1.424	1.440	1.426	1.431	1.450	1.434	1.451
C3-C4	1.418	1.424	1.440	1.420	1.423	1.437	1.434	1.451
C1-C9	1.463	1.475	1.478	1.459	1.474	1.475	1.469	1.471
C4-C10	1.463	1.475	1.478	1.460	1.473	1.477	1.469	1.471
C8-C9	1.407	1.406	1.409	1.409	1.407	1.410	1.408	1.410
C5-C10	1.407	1.406	1.409	1.408	1.406	1.409	1.408	1.410
C5-C6	1.372	1.370	1.385	1.371	1.369	1.386	1.369	1.385
C7-C8	1.372	1.370	1.385	1.371	1.370	1.386	1.369	1.385
O-C1-C2	-	123.5	123.5	-	123.5	122.9	123.3	122.8
O-C4-C3	-	123.5	123.5	-	123.5	123.1	123.3	122.8
C2-C1-C9	-	115.3	115.1	-	116.1	116.0	116.4	116.3
C3-C4-C10	-	115.3	115.1	-	115.3	115.2	116.4	116.3
C1-C2-C3	-	123.3	123.4	-	121.5	121.4	122.4	122.4
C4-C3-C2	-	123.3	123.4	-	124.6	124.7	122.4	122.4
C1-C9-C8	-	119.5	119.5	-	119.3	119.4	119.6	119.7
C4-C10-C5	-	119.5	119.5	-	119.8	119.8	119.6	119.7
C1-C9-C10	-	121.4	121.5	-	121.7	121.7	121.2	121.2
C4-C10-C9	-	121.4	121.5	-	120.9	121.0	121.2	121.2
C7-C8-C9	-	121.0	121.1	-	120.9	121.1	120.9	121.0
C6-C5-C10	-	121.0	121.1	-	120.9	121.0	120.9	121.0
C5-C6-C7	-	119.9	119.9	-	119.8	119.8	119.9	119.9
C8-C7-C6	-	119.9	119.9	-	120.0	119.9	119.9	119.9

^a Atom numbering is consistent with Figure 2-1.

predicts a C=O bond distance approximately 0.01Å longer than those predicted by B3LYP/6-31G(d), which, at about 1.265Å, is about 0.02Å greater in length than that predicted with the UHF/6-31G(d) method. This difference is not repeated in other bonds, implying that the inclusion of polarization functions in the basis set plays an important role in the prediction of the C=O fragment geometries in radical forms of quinones. As with the neutral species, the structure of 23NQ^{•-} is virtually identical to that of MQ^{•-}.

A comparison of the structures of the neutral quinones in Table 2-1 with the associated semiquinone anions in Table 2-2 indicates that the primary changes in bonding upon one-electron reduction are centered in the quinonoidal ring. C=O and C2=C3 bond lengths increase by about 0.04Å, regardless of the method, while C1-C2 and C3-C4 bond lengths decrease by almost the same amount. The shared C9-C10 bond increases in length by about 0.01Å, as do the adjacent C8-C9 and C5-C10 bonds and the more distant C6-C7 bond, while the other two fused ring C-C bonds decrease in length by almost the same amount. These changes in bonding suggest that while the odd electron enters a molecular orbital very similar to that in the parent PBQ molecule,¹⁰³ there is nevertheless a small contribution from the fused ring (see Figure 2-2).

When considering that the fused ring has an effect on the LUMO of the neutral molecule which is, although small, greater than the effect of two substituted methyl groups at the 2- and 3- positions, it is natural to examine the effect of changing these methyl carbons into members of a second fused ring, thus creating a larger π -system. Therefore we include in Table 2-3 a comparison of the calculated structures of 9,10-anthraquinone (AQ) and its associated radical anion (AQ^{•-}) with experiment.^{67,71} Here, B3LYP does an excellent job of reproducing

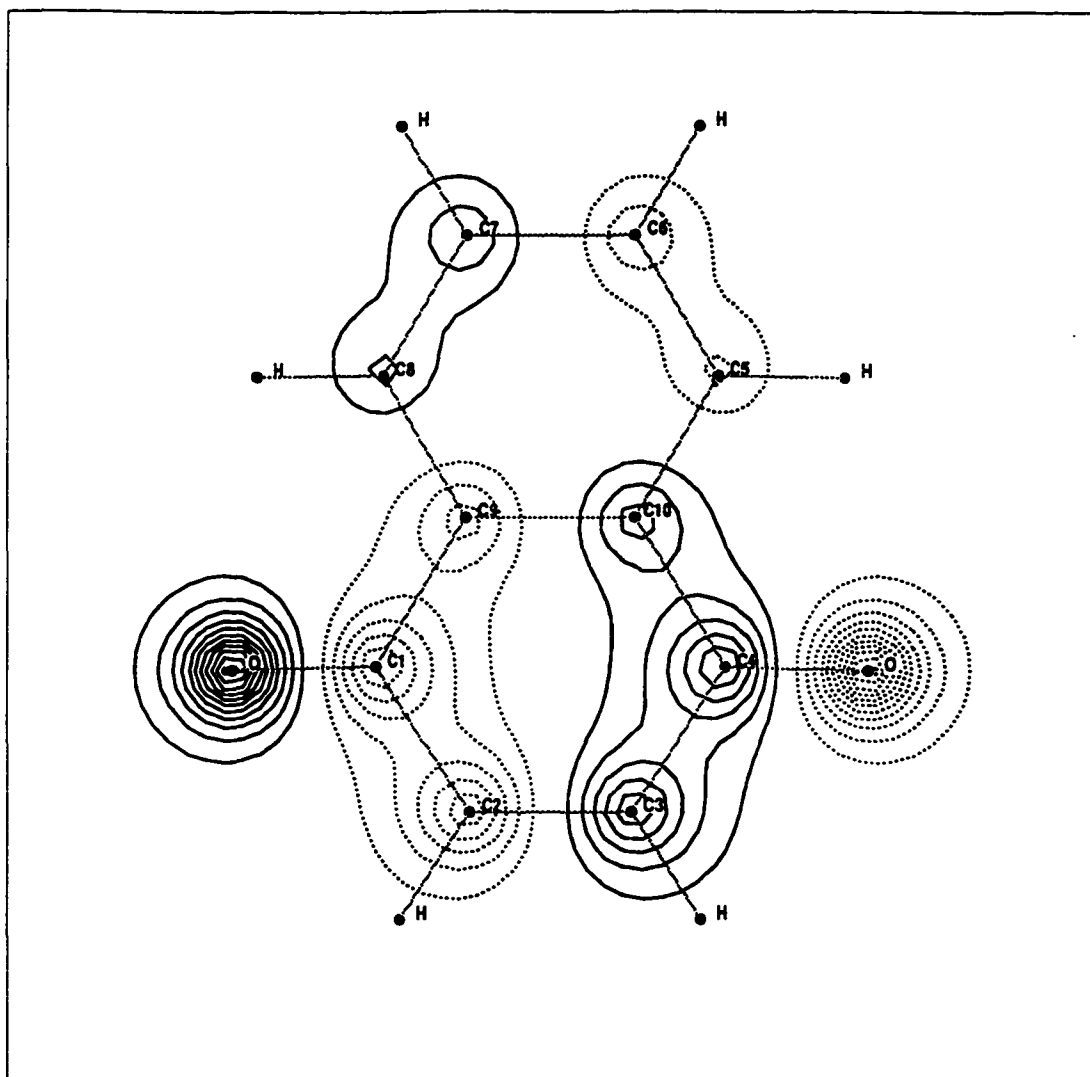


Figure 2-2. The LUMO of 1,4-naphthoquinone. The solid and dashed lines represent orbital lobes of opposite signs.

Table 2-3. Calculated and Experimental Bond Distances (Å) of 9,10-Anthraquinone (AQ) and its Radical Anion (AQ^{-•}).

	AQ					AQ ^{-•}	
	Expt ⁷¹ Gas Phase	Expt ⁶⁷ X-ray	UHF 6-31G(d)	BLYP ¹⁰⁴ 6-31G(d)	B3LYP 6-31G(d)	UHF 6-31G(d)	B3LYP 6-31G(d)
C9=O ^a	1.220	1.15	1.197	1.242	1.226	1.239	1.260
C9-C11	1.499	1.50	1.493	1.501	1.492	1.454	1.465
C11-C1	1.400	1.39	1.389	1.411	1.400	1.415	1.414
C1-C2	1.400	1.39	1.382	1.402	1.392	1.363	1.382
C2-C3	1.400	1.385	1.388	1.411	1.400	1.413	1.415
C11-C12	1.400	1.395	1.393	1.421	1.408	1.409	1.427
C1-H	1.087	-	1.072	1.092	1.085	1.074	1.086
C2-H	1.087	-	1.075	1.094	1.086	1.078	1.089
Average Deviation from Gas Phase Expt.	-	-	0.011	0.010	0.003	-	-
O-C9-C11	121.3	121	121.2	121.3	121.3	122.0	122.0
C11-C9-C14	117.4	117.5	117.7	117.5	117.5	116.0	116.0
C9-C11-C12	121.3	120	121.2	-	121.3	122.0	122.0
C1-C11-C12	120.1	120	119.9	119.7	119.7	118.9	122.0
C11-C1-C2	119.8	120.5	120.0	120.2	120.2	121.2	121.3
C1-C2-C3	119.8	120.8	120.2	-	120.1	120.0	119.9
C11-C1-H	120.1	-	118.9	-	118.3	117.6	117.0
C1-C2-H	120.1	-	119.9	119.9	119.9	120.2	120.2
Average Deviation from Gas Phase Expt.	-	-	0.3		0.4	-	-

^a Atom numbering is consistent with Figure 2-1.

bond distances and angles of the neutral, gas-phase molecule, with average absolute deviations of 0.003 Å and 0.4°, respectively, compared to the deviations in the UHF calculations of 0.011 Å and 0.3°. The structures of both the neutral and radical anion show excellent agreement with the analogous parameters of 23NQ, as do the trends in bonding changes upon one-electron reduction. Previous calculations by Ball et al.¹⁰⁴ on AQ using the gradient-corrected pure density functional method BLYP (see Table 2-3) gave substantially poorer agreement with experiment than did B3LYP. BLYP overestimated the C=O bond lengths in particular by more than 0.02 Å, which is similar to the 0.019 Å overestimation of C=O bonds by the BP86 method in NQ.⁸⁵ These results indicate that hybrid HF/DF methods such as B3LYP are more reliable than gradient-corrected pure density functional methods such as BP86 and BLYP for predicting the structures of quinones.

2.2.2 Molecular geometries of MQ and its radical anion

Comparing the calculated geometries of MQ and MQ^{•-} (Table 2-4) demonstrates that the major structural changes upon reduction are concentrated in the quinonoidal portion of the head group. The C=O and C2=C3 double bonds each lengthen by 0.04 Å and 0.03 Å respectively. The C1-C2 and C3-C4 bonds each shorten by about 0.05 Å, while the C1-C9 and C4-C10 bonds shorten by almost 0.02 Å. The C9-C10, C5-C10, C8-C9, and C6-C7 bonds all lengthen by about 0.01 Å upon reduction, while the C5-C6 and C7-C8 bonds shorten by almost the same amount. C-H bond distances lengthen by less than 0.003 Å throughout the molecule upon reduction. These changes in bonding are consistent with the odd electron entering a molecular orbital with a nodal structure similar to that

Table 2-4. Selected Calculated Bond Distances (Å) and Bond Angles (deg) of Menaquinone (MQ) and its Radical Anion (MQ^{•-}).

	MQ	MQ ^{•-}
C1=O ^a	1.228	1.264
C4=O	1.229	1.266
C2-C3	1.358	1.391
C9-C10	1.404	1.419
C6-C7	1.399	1.411
C1-C2	1.498	1.453
C3-C4	1.493	1.448
C1-C9	1.489	1.473
C4-C10	1.488	1.470
C8-C9	1.398	1.410
C5-C10	1.398	1.410
C5-C6	1.393	1.385
C7-C8	1.393	1.385
O-C1-C2	120.6	122.7
O-C4-C3	120.3	122.0
C2-C1-C9	118.7	116.6
C3-C4-C10	118.6	116.6
C1-C2-C3	120.7	121.8
C4-C3-C2	121.4	122.8
C1-C9-C8	119.8	119.6
C4-C10-C5	119.9	119.9
C1-C9-C10	120.3	121.3
C4-C10-C9	120.1	120.9
C7-C8-C9	119.9	121.0
C6-C5-C10	119.9	120.9
C5-C6-C7	120.2	119.9
C8-C7-C6	120.2	119.9

^a Atom numbering is consistent with Figure 2-1.

which has been described above for NQ (Figure 2-2), implying that the isoprenoid chain has little effect on the LUMO of NQ. Bond angles are predicted to change only slightly upon reduction of MQ, the largest changes being occurring around the carbonyl carbon, but even these differences are less than about 2°. In the optimized structures, the substituent chain has a C2-C3-C12-C13 dihedral of 269.0° in the neutral and 264.0° in the anion, and a C2-C12-C13-C14 dihedral angle (measured counterclockwise looking down the C12-C13 bond) of 238.9° and 236.7°, respectively.

The structures of menaquinone (modeled by 2NQ) and its radical anion were predicted recently using Hartree-Fock calculations and a 6-31G basis set.⁸⁶ The predicted bond lengths for both 2NQ and 2NQ^{•-} in this study were generally shorter (by about 0.01 Å) than those predicted here with the B3LYP/6-31G(d) method. However, the changes in bonding upon reduction are very similar, with the largest difference being that the HF calculations predict a larger change in C=O bond length (by about 0.01) than our calculations. Because of the past successes of hybrid HF/DF methods in predicting the structures of quinones discussed earlier, and the inclusion of the isoprenoid chain (MQ vs. 2NQ), we believe our predicted structures are more accurate.

2.2.3 Spin Properties

It is possible to study the electron transfer to a quinone and its hydrogen bonding environment *in vivo* for biological systems such as the photosynthetic reaction center using electron paramagnetic resonance experiments. Such studies have been performed to investigate the environment of both Q_A and Q_B (the primary and secondary electron-acceptor quinones) in the photosynthetic reaction

center of *Rhodobacter sphaeroides* and photosystem II. The information gathered in these studies provides important information on the orientation and close-contacts of the quinone radicals.

In order to interpret these experiments, it would be helpful to have information on the free molecule as a basis for comparison to accurately determine the effects of the protein environment. Because of the relatively slight changes in geometry when substituting one or two methyl groups or a fused ring, it is interesting to consider whether or not the similarities carry over to the spin properties. We present in Tables 2-5 and 2-6 calculated spin density distributions as well as isotropic hyperfine coupling constants (hfcc's) for the heavy atoms and protons of $\text{NQ}^{\cdot-}$, $2\text{NQ}^{\cdot-}$, and $23\text{NQ}^{\cdot-}$ as well as those determined for $\text{AQ}^{\cdot-}$ (Table 2-7) and $\text{MQ}^{\cdot-}$ (Table 2-8). These properties were determined using the B3LYP method and, where possible, three basis sets. The smaller 6-31G(d) basis set has been shown to give reasonable agreement with experimental spin densities when used with the B3LYP method.^{55,56,96-98} In addition, we also employed a basis set developed by Chipman^{105,106} designed specifically to reproduce hfcc's in higher level calculations. This basis set, denoted [632|41], includes more diffuse and polarization functions on heavy atoms, as well as a tighter s-function on hydrogens. Hfcc's are proportional to the spin density at the nucleus, and calculating them accurately may provide a more stringent test of the methods than spin densities derived from population analysis.

Experiments on $\text{NQ}^{\cdot-}$ in various solvents^{35,107,108} have given magnitudes of the H2 and H3 hfcc's which range from 3.22 to 3.32, which is in excellent agreement with our calculated values of 2.9-3.3. For the H6 and H7 protons, our calculations predict hfcc magnitudes of 0.7 to 0.8, which is in reasonable

Table 2-5. Spin Densities Determined for the Radical Anions of 1,4-Naphthoquinone (NQ⁻), 2-Methyl-1,4-Naphthoquinone (2NQ⁻), and 2,3-Dimethyl-1,4-Naphthoquinone (23NQ⁻) Using the B3LYP Method and a Variety of Basis Sets. Methyl hydrogen values are reported as averages.

	NQ ⁻			2NQ ⁻			23NQ ⁻		
	6-31G(d)	6-311G (d,p)	[63241]	6-31G(d)	6-311G (d,p)	[63241]	6-31G(d)	6-311G (d,p)	
O1 ^a	0.26	0.25	0.24	0.25	0.25	0.24	0.26	0.25	
O4	0.26	0.25	0.24	0.25	0.24	0.23	0.26	0.25	
C1	0.07	0.07	0.09	0.08	0.08	0.08	0.08	0.07	
C2	0.12	0.12	0.11	0.10	0.11	0.11	0.10	0.11	
C3	0.12	0.12	0.11	0.11	0.12	0.11	0.10	0.11	
C4	0.07	0.07	0.09	0.06	0.06	0.10	0.08	0.07	
C5	-0.01	0.00	0.00	-0.01	-0.01	-0.01	0.00	0.00	
C6	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	
C7	0.03	0.03	0.02	0.02	0.02	0.02	0.03	0.03	
C8	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
C9	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.05	
C10	0.05	0.05	0.04	0.06	0.06	0.05	0.05	0.05	
H2	-0.01	-0.01	-0.01	-	-	-	-	-	
H3	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-	-	
H5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
H6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
H7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
H8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Me2	-	-	-	-0.01	-0.01	-0.01	-0.01	-0.01	
Me3	-	-	-	-	-	-	-0.01	-0.01	
H(Me2)	-	-	-	0.00	0.00	0.00	0.00	0.00	
H(Me3)	-	-	-	-	-	-	0.00	0.00	

^a Atom numbering is consistent with Figure 2-1.

Table 2-6. Hyperfine Coupling Constants (in Gauss) Determined for the Radical Anions of 1,4-Naphthoquinone (NQ⁻), 2-Methyl-1,4-Naphthoquinone (2NQ⁻), and 2,3-Dimethyl-1,4-Naphthoquinone (23NQ⁻) Using the B3LYP Method and a Variety of Basis Sets. Methyl hydrogen vaules are reported as averages.

	NQ ⁻			2NQ ⁻			23NQ ⁻	
	6-31G(d)	6-311G (d,p)	[632W1]	6-31G(d)	6-311G (d,p)	[632W1]	6-31G(d)	6-311G (d,p)
O1 ^a	-8.3	-5.3	-6.9	-8.4	-5.4	-6.6	-8.4	-5.3
O4	-8.3	-5.3	-6.9	-8.1	-5.1	-6.7	-8.4	-5.3
C1	-0.4	-3.6	-2.6	-1.0	-3.1	-2.0	0.5	-3.0
C2	2.9	-0.2	0.6	4.0	0.0	0.8	2.5	-0.2
C3	2.9	-0.2	0.6	2.8	-0.4	0.1	2.5	-0.2
C4	-0.4	-3.6	-2.6	-0.3	-3.6	-2.8	0.5	-3.0
C5	-0.9	-1.1	-1.1	-0.8	-1.3	-1.3	-0.9	-1.2
C6	0.9	0.3	0.4	0.7	0.5	0.7	0.9	0.3
C7	0.9	0.3	0.4	0.9	0.1	0.2	0.9	0.3
C8	-0.9	-1.1	-1.1	-1.0	-0.9	-1.0	-0.9	-1.2
C9	0.5	-1.0	-0.7	0.5	-1.3	-1.0	0.5	-1.0
C10	0.5	-1.0	-0.7	0.3	-0.7	-0.3	0.5	-1.0
H2	-3.3	-3.0	-2.9	-	-	-	-	-
H3	-3.3	-3.0	-2.9	-3.3	-2.9	-2.6	-	-
H5	-0.1	0.0	-0.1	-0.1	0.1	0.0	-0.1	-0.1
H6	-0.8	-0.7	-0.7	-0.7	-0.8	-0.9	-0.8	-0.7
H7	-0.8	-0.7	-0.7	-0.8	-0.6	-0.6	-0.8	-0.7
H8	-0.1	0.0	-0.1	0.0	-0.2	-0.2	-0.1	-0.1
Me2	-	-	-	-2.1	-1.9	-1.7	-1.52	-1.7
Me3	-	-	-	-	-	-	-1.52	-1.7
H(Me2)	-	-	-	2.3	2.3	2.1	2.3	2.5
H(Me3)	-	-	-	-	-	-	2.3	2.5

^a Atom numbering is consistent with Figure 2-1.

agreement with the experimental range of 0.57 to 0.655. The H5 and H8 protons show the smallest hfcc magnitudes, with experimental values ranging from 0.26 to 0.57, as compared to our calculated magnitudes of 0.1. The experimental ordering of H2 >> H6 > H8 is thus correctly reproduced by our calculations. Good agreement between experiment^{35,107,108} and theory was also found for 23NQ^{•-} and MQ^{•-}. In the case of the asymmetrically substituted 2NQ^{•-}, the results are more ambiguous since the experimental magnitudes of the H5, H6, H7, and H8 proton hfcc's were found to be equal in some solvents. We are aware of no experimentally determined hfcc's for the heavy atoms of these menaquinone analogues. Our calculations are also in agreement with previous experimental results that substitution of alkyl groups has little effect on the spin density distribution.¹⁰⁷ The results presented here also demonstrate significant differences in the heavy atom hfcc's obtained with the smaller 6-31G(d) basis set versus those obtained with the two larger basis sets, but spin densities and proton hfcc's show much less sensitivity to basis set size.

Total atomic spin densities for MQ^{•-} were determined with Mulliken population analysis for the radical anion using the 6-31G(d) and 6-311G(d,p), and as described earlier, the [632|41] basis sets (the results using the [632|41] basis set are given in Table 2-7). The choice of basis set had little effect on total atomic spin densities, and all predict that the unpaired spin is localized almost entirely on the oxygens and quinone ring. In fact, the spin densities on the quinonoidal ring of MQ^{•-} are very similar to those found for NQ^{•-} and its methylated derivatives, which are given in Table 2-5. The comparison demonstrates that the unpaired electron resides largely on the oxygens in both NQ^{•-} derivatives and MQ^{•-}.

Table 2-7. Spin Densities and Hyperfine Coupling Constants (in Gauss) Determined for the Radical Anion of 9,10-Anthraquinone Using the B3LYP Method and a Variety of Basis Sets.

AQ ^{•-} Atom	6-31G(d)		6-311G(d,p)	
	spin	hfcc	spin	hfcc
O9a	0.23	-7.5	0.22	-4.8
C1	-0.01	-1.2	-0.01	-1.5
C2	0.04	1.4	0.04	0.4
C9	0.08	0.8	0.07	-2.6
C11	0.07	0.3	0.07	-0.7
H1	0.00	0.0	0.00	0.0
H2	0.00	-1.1	0.00	-1.0

Table 2-8. Spin Densities and Isotropic Hyperfine Coupling Constants (in Gauss) for MQ^{•-} From the B3LYP Method and Chipman [632|41] Basis Set . Magnitudes of experimental hyperfines, ¹⁰⁸ where available, are given in parentheses.

MQ ^{•-} Atom	Spin Densities	Hyperfines
H (PBQ)	-	-
H5	0.00	-0.11 (0.30)
H6	0.00	-0.68 (0.74)
H7	0.00	-0.72 (0.74)
H8	0.00	-0.07 (0.26)
H11	0.00	2.76 (2.63)
H12	0.00	1.18 (1.32)
H13	0.00	-0.10
H14	0.00	0.03
O1	0.24	-6.67
O4	0.22	-6.23
C1	0.09	-2.24
C2	0.11	1.10
C3	0.09	0.08
C4	0.10	-1.55
C5	0.00	-1.03
C6	0.03	0.37
C7	0.03	0.45
C8	0.00	-1.12
C9	0.03	-0.72
C10	0.05	-0.83
C11	-0.01	-1.91
C12	-0.01	-1.46
C13	0.01	2.17
C14	0.00	0.04

Isotropic hyperfine coupling constants (hfcc's) for $\text{MQ}^{\bullet-}$ are also given in Table 2-8. The calculated proton hfcc's for $\text{MQ}^{\bullet-}$ compare well with the experimental values given,¹⁰⁸ and nearly as well for other experimental hfcc's determined in ethanol¹⁰⁷ and isopropyl alcohol.³⁵ Differences between hfcc's in $\text{NQ}^{\bullet-}$ and $\text{MQ}^{\bullet-}$ are not negligible. The most dramatic differences are found in the ring carbons, which show differences in some cases of around 1 G. Apparently, the unpaired electron of $\text{MQ}^{\bullet-}$ polarizes spin density at the nuclei differently than it does in $\text{NQ}^{\bullet-}$, even though the spin density distributions in the two radicals are very similar.

For the radical anion of 9,10-anthraquinone, $\text{AQ}^{\bullet-}$, Table 2-7 gives a list of the spin densities and hfcc's determined with the B3LYP method and the 6-31G(d) and 6-311G(d,p) basis sets. Here, as in the cases discussed earlier, basis set size has little effect on spin densities, but a marked effect on the magnitudes and signs of the hfcc's. The ^{17}O hfcc calculated with the 6-31G(d) basis set (-7.5 G) shows better agreement with the experimental value of 7.53 G¹⁰⁹ than does that calculated with the larger 6-311G(d,p) basis set (-4.8 G). Experimental values¹¹⁰⁻¹¹⁵ of the proton hfcc at the 1- position range from 0.35 G to 0.550 G, while those of the 2- position range from 0.93 G to 0.97 G. Here, our calculated hfcc's reproduce only the relative ordering of the proton hfcc's: the magnitude of the calculated hfcc for the 2- position (-1.1) agrees well with the experimental values, but the B3LYP method predicts a hfcc of 0.0 G for the 1- position. Other theoretical methods have been used to determine hfcc's for $\text{AQ}^{\bullet-}$, but without greater success.¹¹⁶ To our knowledge, there are no experimental ^{13}C hfcc's available for $\text{AQ}^{\bullet-}$.

From the data in Tables 2-5 and 2-7, it is clear that spin densities and proton hfcc's show little basis set dependence. Indeed, even the replacement of a hydrogen with a methyl group has little effect on spin distribution, which is not surprising since the odd electron is entering a π -type molecular orbital. Heavy atom hyperfine coupling constants show a much larger dependence on the choice of basis set. Values of the oxygen coupling constant are consistently about 20% smaller in magnitude when using the Chipman basis instead of the 6-31G(d) basis, and smaller still using the 6-311G(d,p) basis. It is possible that adding diffuse functions delocalizes too much of the spin away from the nucleus.⁹³ The hfcc's of the C1 and C4 carbons each increase in magnitude with the larger [632|41] basis, but keep the same sign, while the 6-311G(d,p) basis set gives a greater increase in magnitude. The C2 and C3 carbons' hfcc's tend to decrease in magnitude, changing sign for the 6-311G(d,p) basis, while the C9 and C10 carbons, shared by both the quinonoidal and fused rings, tend to remain at about the same magnitude, but change sign with both of the larger basis sets. The C5, C6, C7, and C8 carbons of the fused ring show only a slight change with the larger basis set, as do the ring and methyl protons. We were unable to complete a successful calculation using the [632|41] basis set for either 23NQ⁻ and AQ⁻. It should be noted that the spin contamination (which may be an indicator of the accuracy of the calculation) of the wave functions used to determine the spin properties of the semiquinones, including anthraquinone, were quite low, with an $\langle S^2 \rangle$ value of about 0.76 before spin annihilation^{117,118} and exactly 0.75, the expected analytical value, after. Questions have been raised about the ability of density functional-based methods to reproduce electronic spin properties,¹¹⁹⁻¹²¹ but there is also evidence that DF calculations are less sensitive than MO calculations to spin contamination.¹²²

2.2.4 Vibrational frequencies

A complete listing of the vibrational modes and assignments for NQ and its radical anion determined with the B3LYP/6-31G(d) method is given in Table 2-9, along with the most complete previous experimental and theoretical assignments. For 2NQ, 23NQ, and AQ, only the modes in the spectroscopically important region 1800 cm^{-1} to 1550 cm^{-1} determined with the B3LYP/6-31G(d) method are listed in Table 2-10 along with available experimental data. A complete list of modes and assignments for each molecule and its radical anion determined using both the B3LYP/6-31G(d) and UHF/6-31G(d) methods may be found in the Supplemental Information.

2.2.4.1 Neutral NQ

For the neutral molecule NQ, we find all six C-H stretching modes from 3200 cm^{-1} to 3226 cm^{-1} . The previous experimental studies find these modes in the range of 3020 cm^{-1} to 3085 cm^{-1} , indicating that the frequencies predicted with the HF/DF method are about 5% too high. UHF/6-31G(d) C-H stretching frequencies (after scaling by 0.8929) are found between 2999 cm^{-1} and 3037 cm^{-1} . The B3LYP ordering of the vibrations in this region agrees exactly with that determined by Nonella⁸⁵ using the gradient-corrected pure DF BP86/6-31G(d,p) method, and is very similar to that found experimentally by Singh and Singh,⁷⁶ except that the two highest modes appear to be reversed. Pecile et al.⁷² also found an ordering of the two highest C-H stretching modes similar to that of Nonella, but also assigned the lowest a_1 mode and next-to-lowest b_2 mode in a reversed order. Of the C-H stretching modes identified by Girlando et al.,⁷⁵ four were uncertain.

Table 2-9. A Comparison of Unscaled B3LYP/6-31G(d) Vibrational Frequencies of 1,4-Naphthoquinone (NQ) and its Radical Anion (in parentheses) With the Most Complete Previous Experimental and Theoretical Assignments.

Mode symmetry and description	B3LYP NQ (NQ ^{-•})	Nonella ⁸⁵ BP86/6-31G(d,p)	Balakrishnan ⁸⁶ expt	Girlando ⁷⁵ expt	Pecile ⁷² expt	Singh ⁷⁶ expt
a ₁ C-H str	3226 (3200)	3139		3076	3073	3072
b ₂ C-H str	3224 (3197)	3136		3076	3079	3085
a ₁ C-H str	3218 (3167)	3130		3076	3059	3062
a ₁ C-H str	3208 (3161)	3125		3059	3030	3037?
b ₂ C-H str	3200 (3144)	3112		3076	3063	3020
b ₂ C-H str	3193 (3140)	3111		3059	3030?	2970
b ₂ C=O asym str	1757 (1577)	1675	1667 (1515)	1672	1675	1678
a ₁ C=O sym str	1752 (1655)	1665	1660 (1441)	1662	1663	1663
a ₁ C2-C3 str	1679 (1523)	1614	(1603)	1607	1605	1588
b ₂ C-C str	1649 (1653)	1591	1599	1600	1590	1605
a ₁ C-C str	1629 (1577)	1573	(1537)	1566	1573	1565
a ₁ C-H bend	1521 (1485)	1462	1445	1460	1467	1453
b ₂ C-H bend	1500 (1484)	1446	1407	1432	1456	1380
b ₂ C-H bend	1403 (1433)	1350	1357 (1327)	1370	1378	1335
a ₁ C-C str	1379 (1372)	1373	1323	1328	1330	
a ₁ C-C str	1329 (1251)	1288		1296	1292	1300
b ₂ C-H bend	1316 (1326)	1265		1296	1303	1235
b ₂ C-H bend	1260 (1248)	1213		1224	1160	1220
a ₁ C-H bend	1196 (1182)	1155		1157	1233	1150
a ₁ C-H bend	1172 (1150)	1126		1142	1147	1120
b ₂ C-H bend	1142 (1147)	1102		1116	1117	
a ₁ C-H bend	1078 (1068)	1039		1053	1056	1050
b ₂ C-C-C bend	1077 (1089)	1039		1087	1093	1018
a ₁ Fused ring breathe	1043 (1040)	1012		1012	1021	
a ₂ C-H wag	1028 (939)	983				
a ₂ C-H wag	1015 (980)	970				998
b ₁ C-H wag	995 (965)	954			986	980

Table 2-9 Continued.

Mode symmetry and description	B3LYP NQ (NQ ⁺)	Nonella BP86/6-31G(d,p)	Balakrishnan expt	Girlando expt	Pecile expt	Singh expt
a ₂ C-H wag	924 (878)	886				
b ₁ C-H wag	881 (838)	849			865	914
a ₂ Quinone ring chair	810 (795)	780				
b ₂ C-C-C bend	794 (808)	769		774		
b ₁ C-H wag	786 (772)	753			774	780
b ₂ C-C-C bend	765 (768)	740		758		
a ₁ C-C-C bend	706 (714)	685		693	692	690
a ₂ Fused ring chair	696 (669)	671				
b ₁ Quinone ring boat	614 (628)	590			611	563
b ₂ C=O asym bend	599 (603)	578		598		450
a ₁ C-C-C bend	556 (562)	539		553	559	540
a ₂ Fused ring deform	480 (485)	458				410
b ₂ C-C-C bend	453 (468)	437		448	412	
a ₁ C-C-C bend	452 (464)	440		448	451	
b ₁ Fused ring boat	421 (434)	402			372	470
a ₁ C=O sym bend	375 (365)	360		368		370
a ₂ C=O chair wag	273 (349)	253				258
b ₂ C-C-C bend	268 (269)	255		266	267	
b ₁ butterfly tors	188 (201)	182				
a ₂ Ring-Ring twist	128 (144)	121				202
b ₁ C=O boat wag	88 (117)	90			100	85

In the region 1550 cm^{-1} to 1800 cm^{-1} , we identify five fundamental vibrations of the neutral molecule: two C=O stretches at 1757 cm^{-1} and 1752 cm^{-1} , and three C-C stretches at 1679 cm^{-1} , 1649 cm^{-1} , and 1629 cm^{-1} (see Figure 2-3). Of the C=O stretches, the B3LYP method predicts that the b_2 mode (antisymmetric stretch) is the higher frequency mode, with the a_1 mode (symmetric stretch) only 5 cm^{-1} lower. This ordering is in agreement with the experimental work of Girlando et al., Pecile et al., and Balakrishnan et al.,⁸⁶ as well as the theoretical assignments by Nonella. The theoretical assignments of Balakrishnan et al. using the HF/6-31G method show the same ordering, with a much larger difference between the frequencies (1712 cm^{-1} for the a_1 mode, and 1681 cm^{-1} for the b_2 mode). Using the UHF/6-31G(d) method (Supplemental Information, Table S2-1), we find that these two modes are reversed with respect to experiment, with the symmetric stretch at 1783 cm^{-1} , and the antisymmetric stretch at 1769 cm^{-1} . Singh and Singh observed modes at 1678 cm^{-1} and 1663 cm^{-1} but assigned them to a Fermi resonance doublet, while Brown⁷⁷ found vibrational modes at 1674 cm^{-1} and 1663 cm^{-1} , but was uncertain whether or not these were true fundamentals, the result of a Fermi resonance, or some intermolecular interaction.

With respect to the three C-C stretching modes of the neutral molecule in the range 1550 cm^{-1} to 1800 cm^{-1} , both the B3LYP and UHF methods predict the C2=C3 stretch to be highest in frequency, at 1679 cm^{-1} and 1646 cm^{-1} respectively. Girlando et al., Pecile et al., and Singh and Singh identified this mode at 1607 cm^{-1} , 1605 cm^{-1} , and 1588 cm^{-1} , respectively, while Balakrishnan et al. did not assign this mode experimentally. Nonella's density functional study found the C2=C3 stretching mode at 1614 cm^{-1} . The last two C-C stretches of NQ

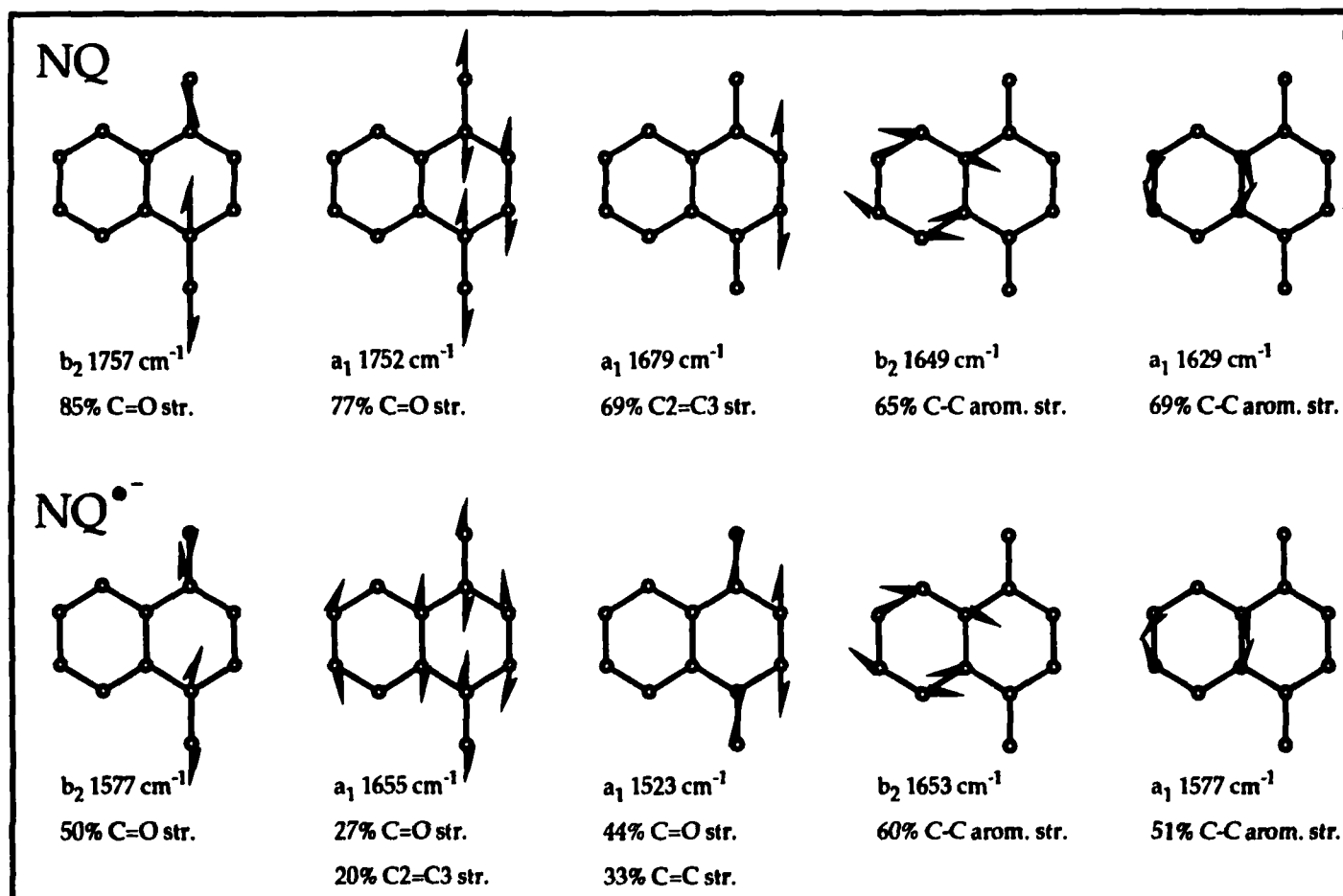


Figure 2-3. Heavy atom Atomic displacements and PED's of the five fundamental vibrational modes (determined with the B3LYP/6-31G(d) method) of naphthoquinone (NQ, top row) in the range 1800-1550 cm^{-1} and the corresponding modes of naphthoquinone radical anion (NQ $^{\bullet-}$, bottom row).

in this frequency range are localized primarily on the fused-ring (see Figure 2-3), with the b_2 mode (1649 cm^{-1}) higher than the a_1 mode (1629 cm^{-1}) according to the B3LYP method and the HF method (at 1603 cm^{-1} and 1582 cm^{-1} , respectively). This is in excellent agreement with previous experimental and theoretical studies. As with the C-H stretching modes, the fundamental vibrations in the range 1550 cm^{-1} to 1800 cm^{-1} predicted by the B3LYP/6-31G(d) method are about 5% too high relative to experiment.

The vibrational spectrum below 1550 cm^{-1} and above 1000 cm^{-1} consists primarily of C-H in-plane bending modes, but also includes three modes which are predominantly C-C stretches, as well as one C-C-C bending mode. The ordering of the modes in this region as predicted by the B3LYP/6-31G(d) method agrees well with the previous experimental assignments with a few exceptions. The a_1 symmetry C-C stretch identified at 1329 cm^{-1} is higher than the b_2 C-H bending mode at 1316 cm^{-1} . While this is in agreement with Nonella and Singh and Singh, it is reversed in relation to the assignments of Pecile et al., and Girlando et al. found these two modes at the same frequency. Also, the a_1 C-H bend and b_2 C-C-C bend predicted at 1078 cm^{-1} and 1077 cm^{-1} appear to be reversed with respect to the experimental assignments of Girlando et al. and Pecile et al., but are in agreement with the work of Singh and Singh, while Nonella predicts them to be found at the same frequency. Pecile et al. assigned the modes at 1233 cm^{-1} and 1160 cm^{-1} to a_1 and b_2 C-H bends, respectively, while all other experimental and theoretical results give reversed assignments, indicating the possibility that these modes were originally misassigned. Nonella, using a gradient-corrected pure density functional method, assigned the modes at 1373 cm^{-1} and 1350 cm^{-1} to an a_1 C-C stretch and a b_2 C-H bend, respectively, which is in disagreement with our

results and the results of all previous experiments. Frequencies in this region are overestimated by the HF/DF method by 2-5% relative to experiment.

Modes with frequencies less than 1000 cm^{-1} are primarily out-of-plane modes, and there are fewer experimental assignments with which to compare our calculated frequencies. All modes within this range are correctly ordered by the B3LYP/6-31G(d) method with respect to the assignments of Girlando et al., and are ordered similarly to those of Nonella except in two cases. The first case is the a_2 C=O chair wag and the b_2 C-C-C bend, predicted by B3LYP to be at 273 cm^{-1} and 268 cm^{-1} , respectively, while Nonella found the order reversed at 253 cm^{-1} and 255 cm^{-1} , respectively. The other disagreement is with the ordering of the a_1 and b_2 C-C-C bends predicted by B3LYP to be virtually identical at 453 cm^{-1} and 452 cm^{-1} , which disagrees with the experimental ordering of Pecile et al. and the theoretical ordering of Nonella, but is consistent with the experimental work of Girlando et al. The only conflict with the ordering of the modes by Singh and Singh in this region is a b_2 C=O bend predicted by B3LYP at 599 cm^{-1} and the a_1 C-C-C bend at 556 cm^{-1} . Singh and Singh assigned these modes in reverse order, which conflicts both with our theoretical study as well as that of Nonella, and with the experimental results of Girlando et al. The overestimation of frequencies in this range by the B3LYP/6-31G(d) method is generally about 3%, which is lower than that found at higher frequencies.

2.2.4.2 $\text{NQ}^{\bullet-}$ Radical Anion

The vibrations of the radical anions of naphthoquinone and its methylated derivatives have been only partially investigated with experimental and theoretical methods. To our knowledge, we provide here the first full vibrational analysis of

NQ^{•-}. Balakrishnan et al.⁸⁶ recently reported several vibrational frequencies of NQ^{•-}, and assigned them based on UHF calculations, and Clark and Evans⁷⁴ reported C=O stretching frequencies for both the radical anion and dianionic forms of NQ.

Figure 2-3 is provided as a comparison of the atomic displacements and total energy distributions of the vibrations in the spectroscopically important range of 1800 cm⁻¹ to 1550 cm⁻¹. Three of the assignments in this range are easy to make: the b₂ C=O antisymmetric stretch found at 1757 cm⁻¹ in NQ shifts downward to 1577 cm⁻¹ in the radical anion, and the b₂ and a₁ fused-ring C=C stretches at 1649 cm⁻¹ and 1629 cm⁻¹ in NQ shift to 1653 cm⁻¹ and 1577 cm⁻¹, respectively, in NQ^{•-}. The two a₁ modes at 1752 cm⁻¹ and 1679 cm⁻¹ in NQ, however, are subject to a large degree of mixing, and the assignments become more difficult. When considering the total energy distributions only, it may be preferable to match the NQ^{•-} mode at 1523 cm⁻¹, which is predominantly C=O stretch, with the NQ mode at 1752 cm⁻¹. However, considering that the phase of the C=O and C2=C3 stretching in the NQ^{•-} mode at 1523 cm⁻¹ is reversed with respect to the NQ mode, they become a questionable match. The radical anion mode at 1655 cm⁻¹ has the correct in-phase C=O and C2=C3 stretching to be a match for the NQ mode at 1752 cm⁻¹, but apparently has become mixed to a significant degree with the a₁ mode at 1577 cm⁻¹. Isotopic substitution of the oxygens (with ¹⁸O) shifts the three highest frequencies of the neutral molecule in this range downward by 32 cm⁻¹, 25 cm⁻¹, and 10 cm⁻¹, respectively, while shifting the last two neutral modes in this range, at 1649 cm⁻¹ and 1629 cm⁻¹, downward by only 1 cm⁻¹. The highest shift in the frequencies of the radical anion in this range upon ¹⁸O substitution occurs for the mode at 1523 cm⁻¹, which shifts

downward by 19 cm^{-1} . The next largest shift is for the b_2 mode at 1577 cm^{-1} , which shifts downward by 14 cm^{-1} , followed by the mode at 1655 cm^{-1} which shifts downward by 6 cm^{-1} . The other two modes of NQ^- , at 1653 cm^{-1} and the symmetric $\text{C}=\text{C}$ stretch at 1577 cm^{-1} , shift downward by 2 cm^{-1} and 4 cm^{-1} , respectively.

Although both the total energy distributions and isotopic substitution data tends to support the reverse, we choose to match the mode at 1655 cm^{-1} in the radical anion with the $\text{C}=\text{O}$ symmetric stretching mode at 1752 cm^{-1} in NQ , and the NQ^- mode at 1523 cm^{-1} with the $\text{C}2=\text{C}3$ stretching mode at 1679 cm^{-1} in the neutral molecule because of the agreement in stretching phases. Our preferred assignment at the B3LYP/6-31G(d) level of calculation is supported by vibrational projection analysis^{123,124} (ViPA, see the Appendix), which shows that the NQ^- mode at 1655 cm^{-1} is made up of 71.8% NQ 1752 cm^{-1} mode, the NQ^- mode at 1653 cm^{-1} is 75.5% NQ 1649 cm^{-1} , the b_2 NQ^- mode at 1577 cm^{-1} is 61.7% NQ 1757 cm^{-1} mode, the a_1 NQ^- mode at 1577 cm^{-1} is 83.9% NQ mode at 1629 cm^{-1} , and the NQ^- mode at 1523 cm^{-1} is 68.2% NQ mode at 1679 cm^{-1} . These assignments would suggest that the modes determined experimentally by Balakrishnan et al. at 1603 cm^{-1} and 1441 cm^{-1} be reassigned to $\text{C}2=\text{C}3$ stretching and symmetric $\text{C}=\text{O}$ stretching, respectively. However, if one chooses to dismiss the phase difference of the $\text{C}=\text{O}$ and $\text{C}2=\text{C}3$ stretching found in the a_1 mode at 1523 cm^{-1} of NQ^- as an artifact of the theoretical method, then the isotopic substitution and total energy distribution calculations support the earlier assignments of Balakrishnan et al. We believe, however, that the ViPA results show that isotopic substitution and TED's are inadequate to unambiguously assign normal modes in this instance. In any case, assignment of vibrational modes in

this region is difficult due to the high degree of mixing between the C=C and C=O stretches, and experimentally the spectrum is dominated by the very strong C=O stretching modes with nearby modes appearing only as shoulders.

Our Hartree-Fock calculations (Table S2-1 in the Supplemental Information) using a 6-31G(d) basis set indicate that the b_2 C=O antisymmetric stretch in $NQ^{\bullet-}$ (at 1509 cm^{-1}) is lower than the a_1 C=O symmetric stretch (at 1622 cm^{-1}). Next highest in frequency are the b_2 and a_1 C=C fused-ring stretches at 1614 cm^{-1} and 1531 cm^{-1} , respectively, with the C2=C3 stretch following at 1461 cm^{-1} . This is in agreement with the ordering described earlier for the B3LYP/6-31G(d) method.

With respect to the other vibrational frequencies of $NQ^{\bullet-}$, we find that, for the most part, the ordering is very similar to that of NQ. The C-H stretching modes of $NQ^{\bullet-}$, although all shifted downward by about 50 cm^{-1} , are found in exactly the same order as those of NQ. In the spectral region of 1000 cm^{-1} to 1550 cm^{-1} , which is dominated primarily by C-H in-plane bending and C-C stretches, we find only two cases of mode reordering upon reduction. The a_1 C-C stretch and the b_2 C-H bend found at 1329 cm^{-1} and 1316 cm^{-1} in neutral NQ, reverse position upon reduction, and are found at 1251 cm^{-1} and 1326 cm^{-1} , respectively. Also, the NQ a_1 C-H bending mode at 1078 cm^{-1} and the C-C-C bend at 1077 cm^{-1} are predicted to be reversed in ordering in $NQ^{\bullet-}$ at 1068 cm^{-1} and 1089 cm^{-1} , respectively. Below 1000 cm^{-1} , several cases of reordering may be identified. The largest frequency shifts in this region upon reduction are found for the a_2 C=O chair wag, which shifts upward 76 cm^{-1} , or 28%, with respect to NQ, and the b_1 C=O boat wag, which shifts upward by 29 cm^{-1} , or 33%.

2.2.4.3 2NQ, 23NQ, and their radical anions

Because of limited experimental data and previous theoretical studies, and because of the more extensive discussion of NQ and NQ^{•-} provided in previous sections of this report, we choose to limit our discussion of the vibrational modes of 2NQ, 23NQ, and their radical anions to the spectroscopically observed region of 1550 cm⁻¹ to 1800 cm⁻¹. A complete description of all vibrational modes for each species obtained with the B3LYP/6-31G(d) and UHF/6-31G(d) methods may be found in the Supplemental Information.

2NQ and its radical anion have been the subject of several experimental studies which focused especially on the C=O stretching frequencies. Table 2-10 shows the frequencies and assignments obtained in this work with the B3LYP method, and compares them to the previous theoretical and experimental studies. Our calculations predict that the b₂ C=O antisymmetric stretch of 2NQ is slightly higher in frequency (by about 5 cm⁻¹) than the a₁ C=O symmetric stretch. Of the experimental studies by Baucher,⁸⁸ Meyerson,⁷⁸ and Brown,⁷⁷ none assigned these modes to "antisymmetric" or "symmetric" stretches, instead they generally favored the interpretation that the C=O splitting was the result of a Fermi resonance, or, in the case of Meyerson, that the splitting was the result of independent fundamental vibrations of either the proximal or distal (with respect to the antisymmetric substitution) carbonyl groups. Our work, as well as that of Balakrishnan et al., supports the notion that the carbonyl splitting results from two nearly degenerate fundamental vibrational modes, each involving both carbonyl groups vibrating either in phase with each other (symmetric) or out of phase (antisymmetric). Our work with the B3LYP/6-31G(d) method, and Balakrishnan et al.'s theoretical work

Table 2-10. A Comparison of the Spectroscopically Important Vibrational Modes Calculated Using the B3LYP/6-31G(d) Method for 2NQ, 23NQ, AQ, and Their Associated Radical Anions (in parentheses) With Previous Experimental^a and Theoretical Assignments.

2NQ (2-methyl-1,4-naphthoquinone)							
Mode	B3LYP	RHF ⁸⁶	Balakrishnan ⁸⁶ expt	Clark ⁷⁴ expt	Baucher ⁸⁸ expt	Meyerson ⁷⁸ expt	Brown ⁷⁷ expt
b ₂ C=O asym. str.	1751 (1569)	1707 (1379)	1669 (1505)	1661 (1505)	1664 (1502)	1673.0	1674
a ₁ C=O sym. str.	1746 (1655)	1673 (1424)	1672 (1442)		1626	1665.5	1663
a ₁ C2-C3 str.	1694 (1549)	1639 (1600)	1624 (1605)	1600	1596		
b ₂ C-C arom. str.	1649 (1649)	1604	1599 (1539)				
a ₁ C-C arom. str	1632 (1580)	1581					
23NQ (2,3-dimethyl-1,4-naphthoquinone)							
Mode	B3LYP	Breton ³³ expt					
b ₂ C=O asym. str.	1741 (1560)	1670					
a ₁ C=O sym. str.	1735 (1639)	1662					
a ₁ C2-C3 str.	1674 (1542)						
b ₂ C-C arom. str.	1649 (1649)						
a ₁ C-C arom. str	1633 (1570)						

Table 2-10 Continued.

AQ (9,10-anthraquinone)

Mode	B3LYP	Clark ⁷⁴ expt	Stenman ⁸² expt	Girlando ⁷⁵ expt	Gastilovich ⁸³ expt	Gribov ⁸⁷ expt	Singh ⁷⁶ expt
b _{1u} C=O asym. str.	1756 (1558)	1675 (1496)		1676		1681	1670
a _g C=O sym. str.	1747 (1645)		1666	1673	1677		1675
b _{3g} C-C str.	1650 (1642)	1592		1584	1625	1574	1625
b _{1u} C-C str.	1646 (1655)			1593		1594	1585
a _g C-C str.	1636 (1564)		1597	1603	1594		1595
b _{2u} C-C str.	1625 (1579)			1582			1572

Ⓢ

^a Where experimental studies did not explicitly assign modes, assignments are suggested here. For a more complete discussion, see the text.

using the RHF/6-31G method, predicts the antisymmetric C=O stretching mode to be higher in frequency, while the UHF/6-31G(d) method predicts the reverse.

As far as the overall ordering in this region of the spectrum for 2NQ, our calculated vibrational frequencies agree with experiment that the C=O stretching modes are highest, followed by the C2=C3 stretching mode, followed by the C=C fused-ring stretching modes. This is the same calculated ordering as is found experimentally for NQ. When compared to experiment, the B3LYP/6-31G(d) modes are generally about 5% too high.

For the radical anion 2NQ⁻, there is much less experimental data. However, the data that are available (see Table 2-10) agree that upon one electron reduction, at least one of the carbonyl stretching modes shifts downward by 9% to 10% to around 1505 cm⁻¹. This shift downward by 10% is reproduced well by the B3LYP/6-31G(d) (which, again, consistently overestimates absolute frequencies by about 5%) and UHF/6-31G(d) methods, and less well (19%) by previous work using the RHF/6-31G method.⁸⁶

As with NQ⁻ (see section 2.4.2), we assign the a₁ C=O stretching mode of 2NQ⁻ to a frequency of 1655 cm⁻¹, followed by the C=C fused-ring stretches at 1649 cm⁻¹ and 1580 cm⁻¹, followed in turn by the C2=C3 stretch at 1549 cm⁻¹. Since there is not enough experimental work available to make a proper assessment of the B3LYP method for reproducing the vibrational modes of 2NQ⁻ in this region, the modes and assignments presented here represent predictions.

In the case of 23NQ and its radical anion 23NQ⁻, there are virtually no experimental studies on the vibrational modes with which to compare our results. Breton³³ assigned a mode at 1662 cm⁻¹ in 23NQ to a C=O vibration, and identified, but did not assign, a shoulder at 1670 cm⁻¹. Because such a shoulder is

a common feature of the C=O stretching frequencies, we suggest the assignment of this frequency to the b_2 C=O antisymmetric stretching mode, and the mode at 1662 cm^{-1} to the symmetric C=O stretch. The overall ordering of the modes of 23NQ and 23NQ $^{\cdot-}$ are predicted to be very similar to NQ and NQ $^{\cdot-}$. The UHF/6-31G(d) method again predicts the symmetric C=O stretch of 23NQ to be higher in frequency than the antisymmetric stretch, while both this method and the B3LYP/6-31G(d) method agree on the ordering of modes in the 1550 cm^{-1} to 1800 cm^{-1} range.

2.2.4.4 MQ and its radical anion

Harmonic vibrational frequencies were calculated and assigned to normal modes for both the neutral and radical anion forms of MQ. The complete set of frequencies for both MQ and MQ $^{\cdot-}$ is reported in Table 2-11. For the neutral molecule MQ, the modes highest in frequency are the C-H stretches found between 3000 cm^{-1} and 3250 cm^{-1} . C-H stretching modes for the radical anion are found in the same region, but are all slightly lower in frequency. Next highest in frequency for MQ are the calculated C=O antisymmetric and symmetric stretching modes at 1737 cm^{-1} and 1732 cm^{-1} , respectively, followed closely by a C2=C3 stretch at 1675 cm^{-1} and two fused ring C=C stretches at 1648 cm^{-1} and 1633 cm^{-1} . These calculated frequencies compare well with the experimentally determined^{46,89} C=O mode at 1661 cm^{-1} , the C2=C3 stretch at 1621 cm^{-1} , and the C=C fused ring stretches at 1596 cm^{-1} and 1582 cm^{-1} . The unscaled calculated frequencies for these modes are each too high by about 3.6%, which is consistent with previous studies of quinones using the B3LYP/6-31G(d) method.^{55,57,58,125} In the radical anion, the C=O stretches are reversed and shifted downward (relative

Table 2-11. Calculated Vibrational Frequencies (cm⁻¹) and Mode Assignments for MQ and MQ⁻.

Mode Description	MQ	MQ ⁻	Mode Description	MQ	MQ ⁻
C-H str.	3236	3209	Fused ring breathe	1059	1046
C-H str.	3229	3205	Chain torsion	1043	1053
C-H str.	3226	3203	C-H wag	1013	979
C-H str.	3207	3164	C-H wag	993	964
C-H str.	3192	3143	C-C str.	985	998
C-H str.	3171	3141	C-C str.	969	965
C-H str.	3170	3165	Chain torsion	939	889
C-H str.	3154	3134	C-C-H bend	927	929
C-H str.	3115	3085	C-H wag	922	877
C-H str.	3106	3059	C-C str.	913	907
C-H str.	3066	3034	C-C-C bend	814	827
C-H str.	3050	3009	Quin. ring chair	810	791
C=O asym str.	1737	1561	C-H wag	802	783
C=O sym str.	1732	1640	Quinoid ring boat	740	733
C=C chain str.	1724	1717	Ring torsion	708	695
C2-C3 str.	1675	1544	Fused ring chair	694	683
C-C str.	1648	1649	C=O bend	685	680
C-C str.	1633	1571	C-C-C bend	664	659
H-C-H bend	1528	1535	C-C-C bend	602	596
C-H bend	1525	1490	Fused ring def.	545	545
H-C-H bend	1515	1517	C-C-C bend	509	519
C-H bend	1502	1496	C-C-C bend	468	471
C-C-H bend	1496	1480	Ring torsion	447	434
H-C-H bend	1471	1466	Fused ring boat	434	454
H-C-H bend	1428	1399	C-C-C bend	419	428
C-C str	1381	1378	C=O bend	398	390
C-C-H bend	1357	1420	C-C-C bend	382	380
C-C-H bend	1340	1337	C-C-C bend	346	347
C-C-H bend	1326	1313	C-H wag	321	321
C-C str	1320	1246	C-C-C bend	297	300
C-H bend	1289	1282	C-C-C bend	249	253
C-C-H bend	1267	1273	Ring-Ring tors.	198	211
C-C-H bend	1215	1219	C=O boat wag	158	171
C-H bend	1194	1156	Methyl torsion	129	160
C-C str.	1169	1191	Ring-Ring twist	124	149
C-H bend	1149	1147	Chain torsion	105	115
C-C-C bend	1115	1112	Ring torsion	74	90
C-C-H bend	1086	1080	Chain torsion	49	54
C-C-H bend	1061	1064	Chain torsion	39	42

to those in the neutral molecule) to 1561 cm^{-1} for the antisymmetric stretch and 1640 cm^{-1} for the symmetric stretch, placing them both below the predicted frequency of the C=C stretching mode from the isoprenoid tail at 1717 cm^{-1} , and close to the C2=C3 mode at 1544 cm^{-1} and the C=C aromatic modes at 1649 cm^{-1} and 1571 cm^{-1} . Among the five modes of $\text{MQ}^{\cdot-}$ for which experimental frequencies are available, the calculated modes are each about 5% to high.

A handful of vibrational frequencies of MQ and $\text{MQ}^{\cdot-}$ were recently assigned experimentally based on model compounds 2NQ and $2\text{NQ}^{\cdot-}$.⁸⁶ Although our ordering of the symmetric and antisymmetric C=O stretching modes are different than those identified in this study experimentally for both the neutral and radical anion, we note that the experimental data in this case are from a vitamin K analogue which omits the isoprenoid chain, and that B3LYP/6-31G(d) (see section 2.4.2.3) and UHF/6-31G⁸⁶ calculations of 2NQ also predict the C=O symmetric stretch to be lower in frequency than the antisymmetric stretch. Other than those mentioned above, there is only one other experimental assignment⁸⁸ of the vibrational frequencies of radical anion vitamin K analogues: a C=O stretch for vitamin K₁ and MQ-9 radical anions, found at 1488 cm^{-1} for both species, and two C=O stretches at 1502 cm^{-1} and 1493 cm^{-1} identified in menaquinone-0 radical anion.

Both MQ and $\text{MQ}^{\cdot-}$ show a C=C stretching mode at about 1720 cm^{-1} , and a series of hydrogen bending modes between 1550 cm^{-1} and 1400 cm^{-1} , and in almost every case, the vibrational frequency of the anion is slightly lower than the corresponding neutral frequency. Below these modes are the C-C stretches and C-C-C bends, and the various torsional modes. In general, these vibrations also display the trend of the anion mode being slightly lower in frequency than the

neutral mode, expect for vibrations below about 300 cm^{-1} , where the trend is reversed. None of the vibrations below 1550 cm^{-1} show the 200 cm^{-1} shift in frequency upon reduction found in the characteristically quinonoidal C=O stretching modes. Comparing the C=C and C=O stretches of MQ to NQ, and those of $\text{MQ}^{\cdot-}$ to $\text{NQ}^{\cdot-}$, we see that vibrational frequencies differ by no more than about 20 cm^{-1} . The differences are likely attributable to the effects of the fused ring and isoprenoid substituent.

(It should be noted that the vibrational mode assignments for MQ and its radical anion are slightly different here than in the previously published work¹ because of the subsequent development of the ViPA method of assigning modes (see the Appendix). The assignments presented here are the most accurate based on ViPA results.)

2.2.4.5 AQ and $\text{AQ}^{\cdot-}$

A thorough discussion of the vibrational modes of neutral AQ was recently reported by Ball et al.,¹⁰⁴ and so only a brief comparison of that work using the BLYP method with our results using the B3LYP method is given, followed by a comparison of the neutral and radical anion vibrations. A list of modes for both AQ and $\text{AQ}^{\cdot-}$ found in the range 1550 cm^{-1} to 1800 cm^{-1} is given in Table 2-10, and a complete list of calculated vibrational modes and descriptions for both the B3LYP and UHF methods may be found in the Supplemental Information (Tables S2-6 and S2-7).

Our calculated vibrational frequency ordering agrees with that of Ball et al. with only two minor exceptions. First, we find that the b_{1u} C-C stretch (at 1193 cm^{-1}) and the b_{2u} C-H bend (at 1192 cm^{-1}) are reversed with respect to the

theoretical assignments of Ball et al. and the experimental assignments of Pecile et al.,¹²⁶ but are in agreement with the experimental ordering found by Gazis¹²⁷ and Girlando et al.⁷⁵ However, the close spacing predicted for these two vibrations makes a definitive ordering impossible based on the calculations presented here. Secondly, we find the b_{3u} C-H wag mode to be higher in frequency (at 720 cm^{-1}) than the a_g C-C-C bending mode (at 695 cm^{-1}), whereas Ball et al. predicts them to have identical frequencies. In this case, our ordering agrees with the experimental work of Lehmann et al.⁷⁹ In both our work and Ball et al.'s, the ordering of the C=O stretches (with the antisymmetric stretch slightly higher than the symmetric stretch) agrees with the experimental work by Girlando et al. and Lehmann et al., but disagrees with the assignments of Singh and Singh.⁷⁶

To our knowledge, experimental and theoretical studies of the radical anion $AQ^{\bullet-}$ are extremely limited. Only Clark and Evans⁷⁴ reports such information: a C=O stretching mode of $AQ^{\bullet-}$ at 1496 cm^{-1} . This 11% downward shift of the C=O vibration upon reduction is consistent with the other quinones described above, and the shift is accurately reproduced by our calculations, although both the neutral and anion C=O vibrational frequencies predicted with the B3LYP/6-31G(d) method are 5% and 4% too high, respectively. Our theoretical assignments also predict that the a_g C=O stretching mode of AQ shifts downward upon reduction, but only by 6%, coming very close to the b_{3g} and b_{1u} C-C stretching modes at 1642 cm^{-1} and 1655 cm^{-1} in $AQ^{\bullet-}$. The a_g and b_{2u} modes found at 1636 cm^{-1} and 1625 cm^{-1} in AQ are predicted to shift downward by 4% and 3%, respectively, in the radical anion $AQ^{\bullet-}$.

2.3 Conclusions

We have presented the first full comparison, either experimental or theoretical, of the structures, spin densities, isotropic hyperfine coupling constants, and vibrations of 1,4-naphthoquinone (NQ), 2-methyl-1,4-naphthoquinone (2NQ), 2,3-dimethyl-1,4-naphthoquinone (23NQ), menaquinone (MQ), and 9,10-anthraquinone (AQ) and their radical anions. We have demonstrated that the geometries of the neutral molecules predicted with the B3LYP/6-31G(d) HF/DF method are consistently closer to experiment than those predicted using gradient-corrected pure density functional methods such as BLYP or BP86. Based on this knowledge, we offer predictions of the geometries of the radical anions of each of the species under investigation.

We have also shown that the B3LYP method, when used in combination with a 6-31G(d) basis set, can provide qualitatively correct spin densities of the radical anions of fused ring quinones. When using the larger [632|41] and 6-311G(d,p) basis sets, the spin densities change very little with respect to the smaller basis sets, but the isotropic hyperfine coupling constants, especially those of the heavy atoms, can change substantially. Because of the lack of experimental data, our results leave unanswered the question of whether or not a larger basis set (such as 6-311G(d,p) or the Chipman [632|41] basis set) is necessary to obtain reliable heavy atom hfcc's, although recent work indicates that large basis sets which are not augmented in the core region give worse hfcc's for semiquinones^{55,93} than does the Chipman [632|41] basis set.

Also, we provide the first complete analysis of the vibrational spectra of the radical anions NQ^{•-}, 2NQ^{•-}, 23NQ^{•-}, MQ^{•-}, and AQ^{•-}. The B3LYP/6-31G(d)

method has been demonstrated to provide frequencies which are uniformly 4% to 5% too high with respect to experimental data, in agreement with previous tests, but the ordering of the frequencies is generally excellent. Where data exist, the B3LYP/6-31G(d) method can accurately predict the changes in the vibrational spectra of the simple fused ring quinones upon one-electron reduction. Our calculated vibrational frequencies for neutral MQ agree well with the few previously determined experimental values, although the ordering of the two C=O stretching modes in MQ may be incorrect.

This work is reported to aid the interpretation of experimental data, especially work in the photosynthetic reaction centers involving the replacement of the native quinones with other species. It is also useful in demonstrating the ability of the HF/DF B3LYP method to predict a wide range of molecular properties of quinones and their radical anions.

2.4 Bibliography

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Table S2-1. Calculated UHF/6-31G(d) Vibrational Frequencies and assignments for 1,4-Naphthoquinone and the Corresponding Modes of the Radical Anion. All frequencies have been scaled by 0.89.

Mode		NQ	NQ ^{•-}	Mode		NQ	NQ ^{•-}
48 C-H str.	a1	3037	3023	24 C-H wag	a2	1018	987
47 C-H str.	b2	3036	3022	23 C-H wag	b1	992	975
46 C-H str.	a1	3031	2984	22 Fused ring breathe	a1	991	982
45 C-H str.	a1	3014	2974	21 C-H wag	a2	910	874
44 C-H str.	b2	3014	2964	20 C-H wag	b1	859	810
43 C-H str.	b2	2999	2954	19 Quinone ring chair	a2	781	767
42 C=O sym str	a1	1783	1622	18 C-H wag	b1	772	763
41 C=O asym str	b2	1769	1509	17 C-C-C bend	b2	756	777
40 C2-C3 str	a1	1646	1461	16 C-C-C bend	b2	732	740
39 C-C str	b2	1603	1614	15 Fused ring chair	a2	672	647
38 C-C str	a1	1582	1531	14 C-C-C bend	a1	670	680
37 C-H bend	a1	1472	1426	13 Quinone ring boat	b1	595	611
36 C-H bend	b2	1445	1441	12 C=O asym bend	b2	574	578
35 C-H bend	b2	1355	1385	11 C-C-C bend	a1	529	534
34 C-C str	a1	1289	1160	10 Fused ring deform	a2	465	473
33 C-C str	a1	1204	1240	9 C-C-C bend	b2	433	446
32 C-H bend	b2	1270	1282	8 C-C-C bend	a1	425	441
31 C-H bend	b2	1209	1194	7 Fused ring boat	b1	409	420
30 C-H bend	a1	1131	1050	6 C=O sym bend	a1	360	352
29 C-H bend	a1	1100	1132	5 C=O chair wag	a2	265	350
28 C-H bend	b2	1092	1102	4 C-C-C bend	b2	256	258
27 C-C-C bend	b2	1032	1050	3 Ring-Ring butterfly	b1	182	194
26 C-H bend	a1	1026	1025	2 Ring-Ring twist	a2	120	139
25 C-H wag	a2	1014	918	1 C=O boat wag	b1	73	111

Table S2-2. Calculated B3LYP/6-31G(d) Vibrational Frequencies and assignments for 2-Methyl-1,4-Naphthoquinone and the Corresponding Modes of the Radical Anion.

Mode		2NQ	2NQ ^{•-}	Mode		2NQ	2NQ ^{•-}
57 C-H str.	a'	3227	3201	28 C-Me-H bend	a'	1040	1030
56 C-H str.	a'	3224	3199	27 C-H wag	a''	1014	979
55 C-H str.	a'	3207	3161	26 C-H wag	a''	994	965
54 C-H str.	a'	3196	3145	25 C-Me str.	a'	958	964
53 C-H str.	a'	3192	3140	24 C-H wag	a''	925	876
52 C-H str.	a'	3149	3097	23 C-H wag	a''	922	879
51 C-H str.	a''	3114	3069	22 Qunone ring chair	a''	807	791
50 C-H str.	a'	3059	3024	21 C-C-C bend	a'	803	818
49 C=O asym. str	a'	1751	1569	20 C-H wag	a''	796	780
48 C=O sym. str.	a'	1746	1655	19 C-C-C bend	a'	723	731
47 C2-C3 str.	a'	1694	1549	18 Fused ring chair	a''	708	691
46 C-C str	a'	1649	1649	17 Quinone ring boat	a''	683	661
45 C-C str	a'	1632	1580	16 C-C-C bend	a'	661	662
44 C-H bend	a'	1523	1491	15 C=O asym bend	a'	652	647
43 H-C-H bend	a'	1507	1511	14 C-C-C bend	a'	520	530
42 C-H bend	a'	1500	1487	13 Fused ring deform	a''	487	500
41 H-C-H bend	a''	1497	1499	12 C-C-C bend	a'	464	476
40 C-Me-H bend	a'	1441	1429	11 Fused ring boat	a''	434	447
39 C-H bend	a'	1385	1415	10 C-C-C bend	a'	409	420
38 C-C str.	a'	1377	1375	9 C=O sym bend	a'	381	370
37 C-C str,	a'	1327	1306	8 C=O chair wag	a''	362	385
36 C-H bend	a'	1290	1241	7 C-C-Me bend	a'	301	296
35 C-H bend	a'	1262	1252	6 C-C-C bend	a'	253	255
34 C-H bend	a'	1195	1165	5 Ring-ring butterfly	a''	214	241
33 C-H bend	a'	1187	1190	4 Me pseudo rotation	a''	163	176
32 C-H bend	a'	1134	1141	3 Me pseudo rotation	a''	133	99
31 C-C-C bend	a'	1109	1105	2 Ring-ring twist	a''	126	144
30 C-Me-H bend	a''	1074	1065	1 C=O boat wag	a''	67	88
29 Fused ring breathe	a'	1059	1046				

Table S2-3. Calculated UHF/6-31G(d) Vibrational Frequencies and assignments for 2-Methyl-1,4-Naphthoquinone and the Corresponding Modes of the Radical Anion. All Frequencies have been scaled by 0.89.

Mode		2NQ	2NQ ^{•-}	Mode		2NQ	2NQ ^{•-}
57 C-H str.	a'	3038	3024	28 Fused ring breathe	a'	1007	994
56 C-H str.	a'	3037	3022	27 C-Me-H bend	a''	1002	982
55 C-H str.	a'	3014	2975	26 C-H wag	a''	992	975
54 C-H str.	a'	3014	2968	25 C-H wag	a'	910	872
53 C-H str.	a'	2999	2954	24 C-Me str.	a''	908	917
52 C-H str.	a'	2940	2892	23 C-H wag	a''	904	854
51 C-H str.	a''	2924	2883	22 Quinone ring chair	a''	785	769
50 C-H str.	a'	2868	2837	21 C-H wag	a'	777	764
49 C=O sym. str.	a'	1776	1622	20 C-C-C bend	a''	765	785
48 C=O asym. str.	a'	1762	1503	19 C-C-C bend	a'	689	700
47 C2-C3 str.	a'	1662	1488	18 Fused ring chair	a''	684	666
46 C-C str	a'	1603	1613	17 Quinone ring boat	a''	661	640
45 C-C str	a'	1583	1530	16 C-C-C bend	a'	630	632
44 C-H bend	a'	1474	1434	15 C=O asym bend	a'	624	620
43 H-C-H bend	a'	1448	1456	14 C-C-C bend	a'	495	505
42 C-H bend	a'	1445	1434	13 Fused ring deform	a''	471	485
41 H-C-H bend	a''	1440	1445	12 C-C-C bend	a'	441	455
40 C-Me-H bend	a'	1398	1383	11 Fused ring boat	a''	419	433
39 C-H bend	a'	1340	1365	10 C-C-C bend	a'	388	401
38 C-C str.	a'	1285	1396	9 C=O sym bend	a'	364	355
37 C-H bend	a'	1248	1263	8 C=O chair wag	a''	347	377
36 C-H bend	a'	1215	1198	7 C-C-Me bend	a'	287	284
35 C-H bend	a'	1202	1180	6 C-C-C bend	a'	240	244
34 C-H bend	a'	1135	1142	5 Ring-ring butterfly	a''	204	231
33 C-H bend	a'	1104	1156	4 Me pseudo rotation	a''	164	194
32 C-H bend	a'	1076	1101	3 Me pseudo rotation	a''	140	116
31 C-C-C bend	a'	1061	1064	2 Ring-ring twist	a''	119	139
30 C-Me-H bend	a''	1043	1029	1 C=O boat wag	a''	55	84
29 C-H wag	a'	1017	986				

Table S2-4. Calculated B3LYP/6-31G(d) Vibrational Frequencies and assignments for 2,3-Dimethyl-1,4-Naphthoquinone and the Corresponding Modes of the Radical Anion.

Mode		23NQ	23NQ ^{•-}	Mode		23NQ	23NQ ^{•-}
66 C-H str.	a ₁	3228	3204	33 C-H bend	a ₁	1066	1050
65 C-H str.	b ₂	3226	3202	32 C-Me-H bend	b ₁	1042	1050
64 C-H str.	a ₁	3207	3162	31 C-Me-H bend	a ₁	1036	1025
63 C-H str.	b ₂	3192	3141	30 C-H wag	a ₂	1013	980
62 C-H str.	b ₂	3171	3140	29 C-H wag	b ₁	993	965
61 C-H str.	a ₁	3171	3140	28 C-C-C bend	b ₂	973	991
60 C-H str.	a ₂	3095	3038	27 C-H wag	a ₂	923	876
59 C-H str.	b ₁	3089	3038	26 Fused ring breathe	a ₁	921	912
58 C-H str.	b ₂	3047	3002	25 C-C-C bend	b ₂	819	832
57 C-H str.	a ₁	3044	3006	24 C-H wag	b ₁	805	783
56 C=O asym str.	b ₂	1741	1560	23 Quin. ring chair	a ₂	801	788
55 C=O sym str.	a ₁	1735	1639	22 Quinone ring boat	b ₁	719	698
54 C2-C3 str.	a ₁	1674	1542	21 Fused ring boat	a ₂	701	682
53 C-C str.	b ₂	1649	1649	20 C=O asym bend	b ₂	690	685
52 C-C str.	a ₁	1633	1570	19 C-C-C bend	a ₁	673	673
51 H-C-H bend	b ₁	1535	1542	18 C-C-C bend	b ₂	623	614
50 C-H bend	a ₁	1525	1489	17 Fused ring deform	a ₂	526	530
49 H-C-H bend	a ₂	1520	1526	16 C-C-C bend	a ₁	508	521
48 H-C-H bend	b ₂	1510	1520	15 C=O chair wag	a ₂	436	430
47 H-C-H bend	a ₁	1506	1507	14 Fused ring boat	b ₁	435	454
46 C-H bend	b ₂	1498	1487	13 C=O sym bend	a ₁	426	431
45 C-Me-H bend	a ₁	1435	1414	12 C-C-C bend	b ₂	421	431
44 C-Me-H bend	b ₂	1429	1390	11 C-C-C bend	a ₁	392	391
43 C-C str.	a ₁	1381	1379	10 C-Me bend	b ₂	348	346
42 C-C str.	b ₂	1347	1424	9 C-Me bend	a ₁	310	310
41 C-C str.	a ₁	1321	1250	8 C-Me wag	b ₁	287	294
40 C-H bend	b ₂	1291	1282	7 C-C-C bend	b ₂	252	255
39 C-H bend	b ₂	1217	1220	6 Ring-ring butterfly	b ₁	172	186
38 C-H bend	a ₁	1195	1156	5 Ring-ring twist	a ₂	127	157
37 CC str+CH bd	a ₁	1172	1194	4 Me pseudo rotation	b ₁	119	157
36 C-Me-H bend	b ₂	1140	1129	3 C-Me wag	a ₂	110	124
35 C-H bend	b ₂	1112	1109	2 Me pseudo rotation	a ₂	81	111
34 C-H + C-C-C	a ₂	1078	1077	1 C=O boat wag	b ₁	59	79
bds.							

Table S2-5. Calculated UHF/6-31G(d) Vibrational Frequencies and assignments for 2,3-Dimethyl-1,4-Naphthoquinone and the Corresponding Modes of the Radical Anion. All Frequencies are scaled by 0.89.

Mode		23NQ	23NQ ^{•-}	Mode		23NQ	23NQ ^{•-}
66 C-H str.	a ₁	3039	3028	33 C-H bend	a ₁	1023	1015
65 C-H str.	b ₂	3038	3026	32 C-H bend	a ₂	1017	986
64 C-H str.	a ₁	3014	2975	31 C-Me-H bend	b ₁	1015	1015
63 C-H str.	b ₂	2998	2955	30 Fused ring breathe	a ₁	999	991
62 C-H str.	a ₁	2990	2956	29 C-H wag	b ₁	991	975
61 C-H str.	b ₂	2963	2956	28 C-Me str.+CCC bd	b ₂	929	951
60 C-H str.	b ₁	2924	2852	27 C-H wag	a ₂	909	872
59 C-H str.	a ₂	2924	2846	26 C-Me str.	a ₁	851	861
58 C-H str.	a ₁	2879	2825	25 C-H wag	b ₁	789	771
57 C-H str.	b ₂	2876	2818	24 Quin. ring chair	a ₂	776	761
56 C=O sym str.	a ₁	1765	1609	23 C-C-C bend	b ₂	773	795
55 C=O asym str.	b ₂	1753	1501	22 Quinone ring boat	b ₁	699	673
54 C2-C3 str.	a ₁	1659	1480	21 Fused ring chair	a ₂	676	659
53 C-C str.	b ₂	1603	1614	20 C=O asym bend	b ₂	655	651
52 C-C str.	a ₁	1589	1526	19 C-C-C bend	a ₁	641	642
51 C-H bend	a ₁	1477	1431	18 C-C-C bend	b ₂	591	585
50 H-C-H bend	a ₁	1458	1452	17 Fused ring deform	a ₂	494	511
49 H-C-H bend	b ₁	1451	1478	16 C-C-C bend	a ₁	483	497
48 C-H bend	b ₂	1446	1443	15 Fused ring boat	b ₁	421	439
47 H-C-H bend	a ₂	1444	1464	14 C=O chair wag	a ₂	408	417
46 H-C-H bend	b ₂	1443	1443	13 C=O sym bend	a ₁	404	409
45 C-Me-H bd	a ₁	1403	1380	12 C-C-C bend	b ₂	399	409
44 C-Me-H bd	b ₂	1398	1381	11 C-C-C bend	a ₁	373	409
43 C-Me-H bd	b ₂	1315	1350	10 C-Me bend	a ₁	342	305
42 C-C str.	a ₁	1281	1165	9 C-Me bend	b ₂	318	332
41 C-H bend	b ₂	1245	1242	8 C-Me wag	b ₁	279	280
40 C-C str.	a ₁	1208	1250	7 C-C-C bend	b ₂	236	244
39 C-H bend	b ₂	1166	1169	6 Ring-ring butterfly	b ₁	166	182
38 C-H bend	a ₁	1120	1142	5 Ring-ring twist	a ₂	118	150
37 C-H bend	a ₁	1095	1165	4 C-Me wag	a ₂	109	115
36 CCH+CH bd	b ₂	1091	1084	3 Me pseudo rotation	b ₁	103	144
35 C-H bend	b ₂	1059	1062	2 Me pseudo rotation	a ₂	57	64
34 CCH bend	a ₂	1042	1040	1 C=O boat wag	b ₁	44	74

Table S2-6. Calculated B3LYP/6-31G(d) Vibrational Frequencies and Assignments for 9,10-Anthraquinone and the Corresponding Modes of the Radical Anion.

Mode		AQ	AQ ^{•-}	Mode		AQ	AQ ^{•-}
66 C-H str.	ag	3231	3207	33 C-H wag	au	1014	980
65 C-H str.	b2u	3230	3206	32 C-H wag	b3u	997	969
64 C-H str.	b1u	3228	3205	31 C-H wag	b1g	994	966
63 C-H str.	b3g	3228	3204	30 Fused asym breathe	b2u	958	943
62 C-H str.	ag	3209	3167	29 C-C-C bend	b3g	936	942
61 C-H str.	b2u	3209	3166	28 C-H wag	b2g	928	872
60 C-H str.	b1u	3194	3146	27 C-H wag	au	919	869
59 C-H str.	b3g	3194	3146	26 C-H wag	b3u	828	793
58 C=O asym str.	b1u	1756	1558	25 Quinone ring chair	b2g	818	804
57 C=O sym str.	ag	1747	1645	24 C-C-C bend	b1u	805	811
56 C-C str.	b3g	1650	1642	23 C-H wag	b1g	786	767
55 C-C str.	b1u	1646	1655	22 Fused asym chairs	au	735	735
54 C-C str.	ag	1636	1564	21 C-H wag + Q boat	b3u	720	693
53 C-C str.	b2u	1625	1579	20 C-C-C bend	ag	695	694
52 C-H bend	ag	1526	1507	19 C=O asym bend	b3g	693	687
51 C-H bend	b2u	1517	1482	18 Fused sym chairs	b2g	666	640
50 C-H bend	b3g	1502	1505	17 C-C-C bend	b2u	640	635
49 C-H bend	b1u	1494	1450	16 C-C-C bend	b1u	624	613
48 C-C str.	ag	1383	1382	15 Fused asym rg def.	au	500	506
47 C-C str.	b2u	1373	1379	14 C-C-C bend	ag	481	495
46 C-H bend	b3g	1336	1364	13 Fused asym boats	b1g	454	476
45 C-C str.	b2u	1320	1246	12 C=O chair wag	b2g	435	429
44 C-H bend	b1u	1296	1271	11 C-C-C bend	b3g	422	430
43 C-H bend	b3g	1244	1229	10 Fused sym boats	b3u	413	423
42 C-C str.	ag	1205	1204	9 C=O sym bend	b2u	395	383
41 C-C str.	b1u	1193	1203	8 C-C-C bend	ag	365	376
40 C-H bend	b2u	1192	1149	7 C-C-C bend	b3g	302	301
39 C-C str.	ag	1183	1167	6 Ring system chair	b1g	230	241
38 C-C-C bend	b1u	1118	1116	5 C-C-C bend	b1u	229	231
37 C-C-C bend	b3g	1117	1116	4 Ring system boat	b3u	161	176
36 C-C str.	b2u	1068	1046	3 Fused sym twist	b2g	132	157
35 Fused sym br.	ag	1061	1043	2 Fused asym twist	au	114	123
34 C-H wag	b2g	1015	983	1 Ring system butterfly	b3u	50	62

Table S2-7. Calculated UHF/6-31G(d) Vibrational Frequencies and assignments for 9,10-anthraquinone and the Corresponding Modes of the Radical Anion. All Frequencies are scaled by 0.89.

Mode		AQ	AQ ^{•-}	Mode		AQ	AQ ^{•-}
66 C-H str.	ag	3043	3026	33 Fused sym breathe	au	1009	984
65 C-H str.	b2u	3043	3026	32 C-H wag	b3u	995	979
64 C-H str.	b1u	3041	3024	31 C-H wag	b1g	991	977
63 C-H str.	b3g	3041	3024	30 Fused asym breathe	b2u	915	862
62 C-H str.	ag	3016	2977	29 C-C-C bend	b3g	905	860
61 C-H str.	b2u	3016	2976	28 C-H wag	b2g	904	880
60 C-H str.	b1u	3001	2957	27 C-H wag	au	897	907
59 C-H str.	b3g	3001	2957	26 C-H wag	b3u	811	778
58 C=O asym str.	b1u	1763	1607	25 Quinone ring chair	b2g	797	777
57 C=O sym str.	ag	1755	1516	24 C-C-C bend	b1u	772	756
56 C-C str.	b3g	1604	1640	23 C-H wag	b1g	767	773
55 C-C str.	b1u	1600	1621	22 Fused asym chairs	au	708	713
54 C-C str.	ag	1587	1513	21 C-H wag + Q boat	b3u	701	671
53 C-C str.	b2u	1576	1522	20 C-C-C bend	ag	662	663
52 C-H bend	ag	1474	1451	19 C=O asym bend	b3g	660	660
51 C-H bend	b2u	1471	1423	18 Fused sym chairs	b2g	643	619
50 C-H bend	b3g	1448	1472	17 C-C-C bend	b2u	611	602
49 C-H bend	b1u	1438	1412	16 C-C-C bend	b1u	597	588
48 C-C str.	ag	1291	1347	15 Fused asym def.	au	484	490
47 C-C str.	b2u	1287	1422	14 C-C-C bend	ag	454	473
46 C-H bend	b3g	1252	1233	13 Fused asym boats	b1g	437	460
45 C-C str.	b2u	1212	1418	12 C=O chair wag	b2g	423	417
44 C-H bend	b1u	1197	1058?	11 C-C-C bend	b3g	405	425
43 C-H bend	b3g	1193	1178	10 Fused sym boats	b3u	400	409
42 C-C str.	ag	1147	1290	9 C=O sym bend	b2u	378	365
41 C-C str.	b1u	1138	1157	8 C-C-C bend	ag	343	358
40 C-H bend	b2u	1098	1110	7 C-C-C bend	b3g	291	300
39 C-C str.	ag	1098	1154?	6 Ring system chair	b1g	219	229
38 C-C-C bend	b1u	1069	1073	5 C-C-C bend	b1u	217	221
37 C-C-C bend	b3g	1068	1092	4 Ring system boat	b3u	154	170
36 C-C str.	b2u	1018	1007	3 Fused sym twist	b2g	122	152
35 C-H wag	ag	1018	988	2 Fused asym twist	au	110	120
34 C-H wag	b2g	1017	986	1 Ring system butterfly	b3u	39	59

CHAPTER 3

A Hybrid Hartree-Fock/Density Functional Investigation of the Vibrational Spectra of *para*-Quinones in Various Reduced, Protonated, and Hydrogen-Bonded States

3.1 Introduction

Quinones act as essential electron and proton acceptors in both plant and bacterial photosynthesis.¹ A complete understanding of how they function is an important part of understanding the process of photosynthesis itself. In bacterial photosynthetic reaction centers (RC), which are used as models of plant photosystems² since their three-dimensional structures³⁻⁷ are known, two types of quinones are found naturally: ubiquinone and menaquinone. In the RC of *Rhodospseudomonas viridis*, a ubiquinone acts as the primary electron acceptor, Q_A, while a menaquinone acts as the secondary electron acceptor, Q_B. In *Rhodobacter sphaeroides*, ubiquinone is used as both the primary and secondary electron acceptor. Both bacterial reaction centers are nearly symmetric about a C₂ axis, but electron transport normally proceeds through only one of the two nearly equivalent sides. The primary quinone, Q_A, accepts a single electron at a time, and passes it on to Q_B, while Q_B accepts two electrons and two protons to form a hydroquinone, and then moves out of the reaction center and is replaced by a new quinone to begin the cycle again.⁸ In this manner a proton gradient is formed which drives the synthesis of other important biomolecules such as ATP.

Understanding how identical quinones can function quite differently on different sides of the reaction center is an important goal of current photosynthetic research. One of the most common methods used to study the binding and redox reactions of quinones *in vivo* is vibrational spectroscopy.⁹⁻²³ The C=O and C=C stretching bands of quinones, usually in the range 1700-1200 cm⁻¹, are known to be redox sensitive and are often used to follow the process of quinone reduction. However, the spectra can be difficult to interpret because of complications due to nearby protein and chlorophyll/pheophytin vibrational modes.^{19,20,24-26} In addition, hydrogen bonding to quinone species, which is known to affect redox behavior,^{27,28} can also affect the vibrational frequencies.¹⁰⁻¹⁶ With this study, we provide the first complete description of the vibrational spectra of all the methyl-substituted derivatives of 1,4-benzoquinone (PBQ) and their radical anions, as well as the dianionic, protonated anion, neutral radical, and hydroquinone forms of three representative quinones: 1,4-benzoquinone, 2,3,5-trimethyl-1,4-benzoquinone (TMQ), and 1,4-naphthoquinone (NQ). In addition, we present the first computational investigation of the effects of hydrogen bonding on the vibrational spectra of the parent PBQ molecule and its radical anion and dianionic forms (see Figure 3-1 for representative geometries and numbering schemes). This information should provide a useful reference when interpreting future experimental spectra from photosynthetic reaction centers.

The structure and vibrational spectra of PBQ has been thoroughly investigated in a number of studies.^{29-39,90} The radical anion of PBQ, PBQ^{•-}, has recently been characterized by a variety of techniques to determine its vibrational³⁷⁻⁴⁵ and spin properties,⁴⁶⁻⁵⁵ and theoretical methods have had a great deal of success reproducing experimental findings. Properties of other, larger

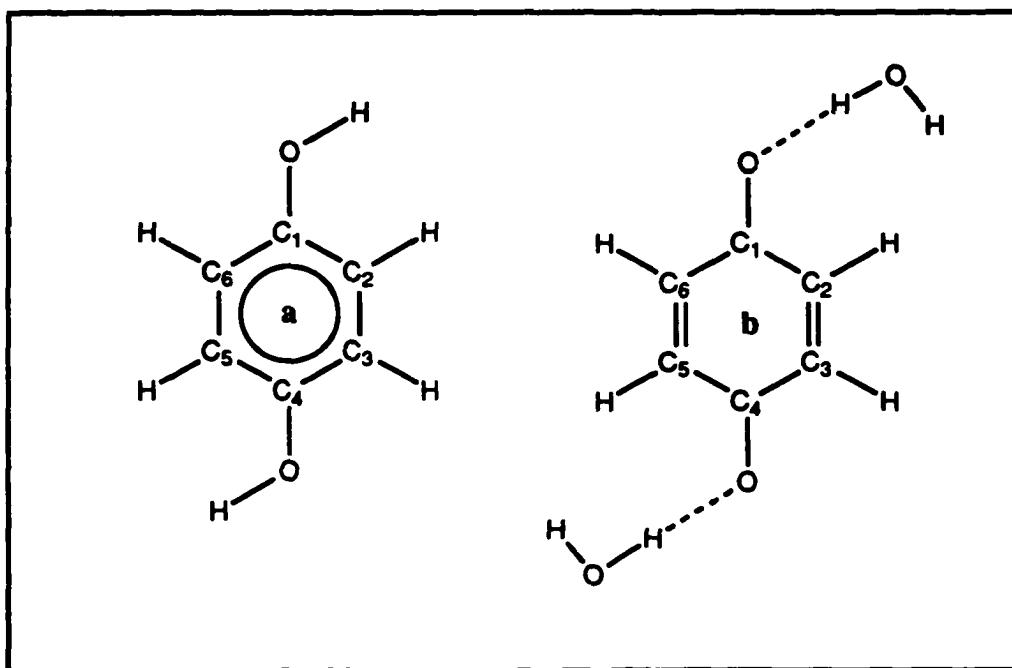


Figure 3-1. Protonated and Hydrogen Bonded Quinones. After accepting two electrons and two protons in photosynthetic reaction centers, a protonated quionone, or hydroquinone, such as the para-hydroquinone *a* shown above, leaves the reaction center. While in the reaction center, the quinones may be hydrogen bonded to amino acids, similar to model *b*, where the hydrogen bond donors are water.

quinones such as plastoquinone, ubiquinone, menaquinone, and model compounds have only recently been examined extensively with computational techniques.^{37,48,50,56-59} Computationally efficient density functional (DF) and hybrid Hartree-Fock/density functional (DF/HF) methods have been widely successful in these and other studies of quinones designed to reproduce or predict a variety of properties including not only structural and spectroscopic features, but also thermodynamic properties.^{37,39,60-64} We tested the ability of a variety of computational methods to reproduce the gas-phase structure of a typical methyl-substituted quinone, 2,3,5,6-tetramethyl-1,4-benzoquinone, or duroquinone (DQ). Based on these tests we selected the HF/DF method B3LYP/6-31G(d) to perform the calculations described here. Because the structural and spin properties of reduced, protonated, and hydrogen bonded quinones have been thoroughly investigated by others using both experimental and computational methods,^{28,50,65-74} we concentrate here on the vibrational spectra of the various quinone derivatives, although some discussions of calculated geometries are provided.

Geometry optimizations and frequency calculations have been performed for all six molecules in the series of methyl-substituted quinones: 2,3,5,6-tetramethyl-1,4-benzoquinone or duroquinone (DQ), 2,3,5-trimethyl-1,4-benzoquinone (TMQ), 2,6-dimethyl-1,4-benzoquinone (26Q), 2,5-dimethyl-1,4-benzoquinone (25Q), 2,3-dimethyl-1,4-benzoquinone (23Q), and methyl-1,4-benzoquinone (2Q). We also performed similar calculations for the radical anions of each of these species. Next, because PBQ and its radical anion provide good structural and spectroscopic models for other *para*-quinones in general, we present calculated geometries and vibrational frequencies for its dianionic (PBQ²⁻), protonated anion (PBQH⁻), hydroquinone (PBQH₂), and neutral radical (PBQH[•])

forms. To further model asymmetrically substituted and fused ring quinones (such as plastoquinone, ubiquinone, and menaquinone) we also provide the results of similar calculations on the reduced and protonated forms of TMQ and NQ. We then use PBQ in the neutral, radical anion, and dianionic forms hydrogen-bound to one or two waters to determine the effects of strong hydrogen bonding on the important vibrational bands. For each case we compare our results to previous theoretical and experimental studies.

In order to compare the vibrational spectra between similar molecules properly, we use the vibrational projection analysis (ViPA) method, described in more detail in Chapter 1. The vibrations of each reduced, protonated, or hydrogen bound species are assigned based on the vibrations of the parent species. Total energy distributions⁷⁵ (TEDs) and isotopic substitutions calculations are used where appropriate, but they are not used to assign vibrational modes. In some cases, this may result in our description of a particular vibration differing from previous descriptions. For instance in PBQ, a particular mode may be 75% C=O stretch by TED analysis, but the matching vibrational mode in PBQ²⁻ may be 60% C=C stretch. (For simplicity of description, we refer to C=C and C=O bonds in reduced and protonated species though strictly they may be closer to aromatic C-C and single C-O bonds than the strict double bonds present in PBQ.) We would then describe both modes as a C=O stretch, because ViPA clearly establishes their similarity. We believe that it is important to show how modes change with reduction (or protonation, etc.) and that this is most easily accomplished by basing our mode descriptions on those of the parent molecule, and not on TED descriptions which are specific to the molecule and may not always provide correct matches between similar molecules.⁷⁶ Therefore, although our mode

descriptions may differ from previous studies, it is simply because we compare them in a different, and more quantitative way.

3.2 Results and Discussion

3.2.1 Method Selection

In order to justify the selection of a method for the prediction of gas-phase geometry of quinones, we tested the ability of several local and gradient corrected density functional methods, the unrestricted Hartree-Fock method, and two hybrid Hartree-Fock/density functional methods to reproduce the gas phase structure of DQ. Duroquinone was chosen because it is the only methyl-substituted PBQ for which an experimental gas phase structure²⁹ was available in addition to a solid-phase X-ray diffraction structure.⁷⁷ The results are shown in Table 3-1, along with the average absolute deviation from the electron diffraction structure for each method. The B3LYP/6-31G(d) method reproduces bond angles almost as well as any other method, but it gives bond distances much closer to experiment than any other method. These results are consistent with previous studies of PBQ derivatives, where HF/DF methods were very successful.^{28,37,44,48,59,90} It should be noted that we performed geometry optimizations at the 6-311G(d,p) and 6-311G(3d,p) level with the B3LYP method (for electron affinity calculations⁶² discussed in Chapter 4), but the structures were slightly less accurate than those obtained with the 6-31G(d) basis set. We therefore elected to use the B3LYP/6-31G(d) method for the rest of the structure and vibrational frequency calculations emphasized here. It is interesting to consider cases where smaller, more computationally efficient basis set may give better results, since the ability to

Table 3-1. A Comparison of the Calculated Bond Lengths (Å) and Bond Angles (Deg) of Duroquinone (DQ) With Experiment. All calculations were performed with the 6-31G(d) basis set.

Structural Parameter	Exptl. Gas Phase ²⁹	B3LYP	UHF	BLYP	BP86	BPW91	BVWN	SLYP	SVWN	B3P86	Exptl. X-ray ⁷⁷
C=O	1.229	1.228	1.197	1.245	1.243	1.242	1.243	1.235	1.233	1.226	1.232
C-C	1.491	1.494	1.498	1.503	1.497	1.497	1.505	1.473	1.475	1.488	1.492
C=C	1.352	1.353	1.331	1.367	1.366	1.365	1.366	1.356	1.354	1.351	1.341
C-Me	1.504	1.504	1.506	1.514	1.506	1.506	1.518	1.475	1.479	1.496	1.514
C-H	1.102	1.095	1.082	1.103	1.104	1.102	1.096	1.112	1.105	1.095	0.97
C-C=C	119.6	102.2	119.6	120.2	120.2	120.2	120.2	120.1	120.1	120.2	120.15
C=C-Me	124.3	122.8	126.6	122.8	122.8	122.8	122.8	122.9	122.9	122.8	122.94
C-C-Me	116.1	117.0	113.8	117.0	117.0	117.0	117.0	117.0	117.0	117.0	116.91
C-C-H	110.5	110.9	110.9	110.9	110.8	110.8	110.9	110.6	110.7	110.7	110.8
H-C-H	-	108.1	107.8	108.1	108.1	108.1	108.0	108.4	108.2	108.1	116.9
Avg. Abs. Bond Dev. from Gas Phase	-	0.002	0.016	0.011	0.007	0.007	0.012	0.013	0.010	0.004	-
Avg. Abs. Angle Dev. from Gas Phase	-	0.9	1.25	0.9	0.8	0.8	0.9	0.7	0.8	0.8	-

perform calculations on larger systems will be, in part, dependent on the availability of a tractable method.

3.2.2 Methyl-Substituted Quinones and Their Radical Anions

Table 3-2 displays the calculated geometries for the entire series of methyl-substituted quinones and their radical anions. C=O bond distances are predicted to be virtually the same across the entire series for any two species with similar charges, with a maximum difference of 0.005 Å between PBQ and DQ. C-C bonds and C=C bonds differ significantly across the series only when atoms in one bond have been chemically substituted differently than those of another. C-C bonds tend to lengthen by approximately 0.02 Å upon methyl substitution, while C=C bonds tend to lengthen by about 0.01 Å. Bond angles also show a great deal of similarity across the series, with differences usually less than about 2° occurring primarily because of different patterns of substitution, which is consistent with other computational studies of quinones.⁵⁷

Besides DQ, solid state structures are also available for 2Q,⁷⁸ 23Q,⁷⁹ 25Q,⁸⁰ 26Q.⁸¹ The accuracy in reproducing experimental geometries in these cases is about the same as that found in DQ. In the case of 25Q, non-hydrogen-containing bonds are reproduced very well, with a maximum deviation of 0.006 Å occurring in the C-Me bond differences, and an overall average error of about 0.003 Å. Bond angles of 25Q are reproduced to within about 1° or better. The structures of 26Q, 23Q, and 2Q are reported with less precision (bonds are reported only to 0.01 Å) than other experimental structures used here. The maximum deviation in the calculated structures of these molecules from experiment is about 0.02 Å, with angles accurate to within 1°.

Table 3-2. A Comparison of the Calculated Bond Distances (Å) and Angles (Deg) for the Series of Methylated 1,4-Benzoquinones and Their Radical Anions Using the B3LYP/6-31G(d) Method.

	PBQ / PBQ ^{•-39}	2Q / 2Q ^{•-}	23Q / 23Q ^{•-}	25Q / 25Q ^{•-}	26Q / 26Q ^{•-}	TMQ / TMQ ^{•-}	DQ / DQ ^{•-}
C1=O	1.225 / 1.266	1.225 / 1.268	1.227 / 1.269	1.227 / 1.268	1.226 / 1.269	1.228 / 1.269	1.228 / 1.271
C4=O	1.225 / 1.266	1.226 / 1.267	1.227 / 1.269	1.227 / 1.268	1.227 / 1.267	1.227 / 1.270	1.228 / 1.271
C1-C2	1.486 / 1.453	1.500 / 1.462	1.496 / 1.465	1.500 / 1.460	1.498 / 1.458	1.496 / 1.462	1.494 / 1.459
C1-C6	1.486 / 1.453	1.485 / 1.449	1.484 / 1.447	1.479 / 1.449	1.498 / 1.458	1.478 / 1.447	1.494 / 1.459
C4-C3	1.486 / 1.453	1.481 / 1.453	1.496 / 1.465	1.479 / 1.449	1.480 / 1.451	1.496 / 1.461	1.494 / 1.459
C4-C5	1.486 / 1.453	1.487 / 1.451	1.484 / 1.447	1.500 / 1.460	1.480 / 1.451	1.498 / 1.456	1.494 / 1.459
C2-C3	1.343 / 1.372	1.348 / 1.374	1.357 / 1.381	1.347 / 1.374	1.347 / 1.374	1.355 / 1.382	1.353 / 1.379
C5-C6	1.343 / 1.372	1.342 / 1.372	1.340 / 1.370	1.347 / 1.374	1.347 / 1.374	1.345 / 1.372	1.353 / 1.379
C2-X	1.086 / 1.090	1.500 / 1.507	1.504 / 1.510	1.499 / 1.506	1.500 / 1.507	1.504 / 1.509	1.505 / 1.510
C3-X	1.086 / 1.090	1.087 / 1.091	1.086 / 1.510	1.087 / 1.091	1.087 / 1.091	1.506 / 1.510	1.505 / 1.510
C5-X	1.086 / 1.090	1.086 / 1.090	1.504 / 1.090	1.499 / 1.506	1.500 / 1.091	1.500 / 1.507	1.505 / 1.510
C6-X	1.086 / 1.090	1.086 / 1.090	1.086 / 1.090	1.087 / 1.091	1.087 / 1.507	1.087 / 1.090	1.505 / 1.510
O-C1-C2	121.4 / 122.7	121.0 / 121.7	121.6 / 122.5	120.7 / 121.7	120.4 / 121.7	118.6 / 122.5	120.2 / 121.6
O-C4-C3	121.4 / 122.7	121.5 / 122.5	121.6 / 122.5	120.9 / 122.6	121.2 / 122.5	121.1 / 122.5	120.2 / 121.6
C6-C1-C2	117.3 / 114.6	118.2 / 115.5	118.5 / 115.8	118.4 / 115.7	119.2 / 116.6	118.6 / 115.9	119.6 / 116.8
C5-C4-C3	117.3 / 114.6	117.4 / 114.8	118.5 / 115.7	118.4 / 115.7	117.7 / 114.9	119.3 / 116.7	119.6 / 116.8
C1-C2-C3	121.4 / 122.7	119.2 / 121.0	120.4 / 121.7	118.7 / 120.5	119.3 / 121.0	120.0 / 121.3	120.2 / 121.6
C1-C6-C5	121.4 / 122.7	121.5 / 122.8	121.1 / 122.5	122.9 / 123.7	119.3 / 121.0	122.5 / 123.4	120.2 / 121.6
C4-C3-C2	121.4 / 122.7	122.8 / 123.6	120.4 / 121.7	122.9 / 123.7	122.3 / 123.2	120.6 / 121.9	120.2 / 121.6
C4-C5-C6	121.4 / 122.7	120.9 / 122.2	121.1 / 122.5	118.7 / 120.5	122.3 / 123.2	119.0 / 120.7	120.2 / 121.6
C1-C2-X	115.9 / 116.2	121.0 / 116.6	116.8 / 116.5	116.9 / 116.9	116.5 / 116.6	117.0 / 116.8	117.0 / 116.8
C1-C6-X	115.9 / 116.2	115.5 / 116.0	115.8 / 116.2	115.1 / 115.7	116.5 / 116.6	115.2 / 115.8	117.0 / 116.8
C4-C3-X	115.9 / 116.2	115.3 / 115.9	116.8 / 116.5	115.1 / 115.7	115.6 / 116.1	116.7 / 116.5	117.0 / 116.8
C4-C5-X	115.9 / 116.2	116.1 / 116.5	115.8 / 116.2	116.9 / 116.9	115.6 / 116.1	116.8 / 116.8	117.0 / 116.8

Comparing the calculated structures of the neutral and radical anion species in Table 3-2 shows that the geometrical changes that occur upon gaining an electron are virtually identical across the series. C=O bonds lengthen by about 0.04 Å and C=C bonds lengthen by about 0.02 Å. C-C bonds in the ring system shorten by approximately 0.03 Å. Changes in the bond distances within substituted groups are generally negligible, as are the changes in bond angles throughout the molecule, which show differences generally less than 2°. All these changes in geometry upon reduction are consistent with the added electron entering an unoccupied orbital centered mainly on the ring and anti-bonding in C=O and C=C, but bonding in the ring C-C bonds.⁸²

Table 3-3 provides a comparison of the most important calculated vibrational frequencies for both the neutral and radical anion forms of all seven quinones in the methyl-substituted series (and a complete list of all calculated vibrational frequencies and assignments are given in the Supplemental Tables S4-1 through S4-6). Experimental vibrational spectra of some of the methyl-substituted derivatives of PBQ are available elsewhere,^{73,83-88} and since we are primarily concerned with similarities between the molecules, it is important to show that the B3LYP/6-31G(d) method does a good job of reproducing the ordering and absolute values of frequencies, and that PBQ may serve as a model compound. (It has been shown⁸⁹ that the B3LYP/6-31G(d) method consistently overestimates vibrational frequencies by about 4%, which can be applied as a “scaling factor” of 0.9614 if so desired.) To facilitate comparison with experiment we change our convention of reporting only unscaled frequencies and instead report frequencies which have been scaled by 0.9614. All scaled frequencies are explicitly indicated in the text.

Table 3-3. The 18 Non-Substituent Harmonic Vibrational Frequencies (cm^{-1}) of the Series of Methyl-Substituted 1,4-Benzoquinones and Their Radical Anions Determined with the B3LYP/6-31G(d) Method.

Mode	PBQ / PBQ ⁻³⁹	2Q / 2Q ⁻	23Q / 23Q ⁻	25Q / 25Q ⁻	26Q / 26Q ⁻	TMQ / TMQ ⁻	DQ / DQ ⁻
C=O asym str.	1758 / 1574	1752 / 1570	1742 / 1563	1745 / 1562	1743 / 1566	1736 / 1556	1727 / 1546
C=O sym str.	1756 / 1674	1753 / 1674	1742 / 1663	1748 / 1677	1749 / 1675	1741 / 1668	1725 / 1654
C=C sym str.	1693 / 1527	1705 / 1557	1701 / 1519	1712 / 1544	1712 / 1542	1714 / 1541	1708 / 1540
C=C asym str.	1659 / 1520	1669 / 1521	1662 / 1552	1682 / 1571	1681 / 1567	1673 / 1562	1673 / 1556
C-C str.	1406 / 1460	1404 / 1454	1402 / 1449	1388 / 1457	1395 / 1448	1382 / 1451	1352 / 1450
C-C str.	1328 / 1231	1310 / 1224	1324 / 1235	1273 / 1217	1312 / 1261	1332 / 1335	1314 / 1233
CC=C bend	951 / 966	893 / 923	1154 / 1178	1180 / 1166	912 / 945	1215 / 1213	1281 / 1313
Ring chair	798 / 756	792 / 753	792 / 760	787 / 749	795 / 760	789 / 759	787 / 763
Ring breathe	777 / 823	685 / 719	629 / 650	671 / 715	570 / 582	563 / 576	546 / 560
C-C str.	756 / 791	767 / 797	823 / 846	806 / 838	769 / 799	829 / 854	841 / 861
CC=C bend	602 / 632	582 / 591	598 / 602	614 / 617	616 / 622	648 / 649	420 / 435
Ring boat	516 / 524	590 / 598	603 / 616	674 / 670	657 / 646	688 / 680	718 / 707
CC=C bend	455 / 469	440 / 455	468 / 481	401 / 422	447 / 464	471 / 487	443 / 462
CC=O bend	454 / 469	485 / 505	598 / 602	615 / 617	468 / 494	341 / 339	364 / 680
CC=O bend	412 / 390	400 / 383	414 / 469	403 / 383	397 / 380	421 / 401	439 / 425
Ring boat	340 / 397	394 / 427	510 / 507	399 / 434	451 / 482	513 / 518	553 / 557
C=O chair	242 / 329	268 / 341	269 / 347	197 / 247	312 / 355	372 / 390	117 / 157
C=O boat	99 / 138	91 / 131	88 / 127	76 / 104	78 / 116	75 / 102	65 / 96

One change in the previously assigned modes of PBQ and PBQ^{•-} should be noted here. We match the C=O and C=C symmetric stretching modes of PBQ at 1756 cm⁻¹ and 1693 cm⁻¹ with the radical anion modes at 1674 cm⁻¹ and 1527 cm⁻¹, respectively. This is opposite of previous studies from our research group,^{37,39,90} which were done before the development of the vibrational projection analysis method (ViPA, see Chapter 1). ViPA shows that our assignments are each 72% matches, whereas the previous assignments are only 27% matched. The difference is in the phase of the coupling between the C=C and C=O stretches. (A thorough discussion of a similar problem with NQ/NQ^{•-} was presented in Section 2.2.4.2.) Therefore the new assignments should be more appropriate.

For the neutral molecules, our calculations show that the ordering of the ring stretching modes above 1200 cm⁻¹ is similar across the series of methyl-substituted PBQs. C=O stretching modes are predicted to be highest in frequency, followed by C=C stretching modes and two C-C modes. We predict that the C=O antisymmetric stretch occurs at a higher frequency than the C=O symmetric stretch in DQ, just as it is does PBQ (both of which have the same symmetry, D_{2h}), while for all the other, less symmetric molecules, the two C=O stretching modes are at least equal (as in the case of 23Q), or reversed. In all the neutral molecules, the C=C symmetric stretch occurs at higher frequencies (~1710 cm⁻¹) than the C=C antisymmetric stretch (~1670 cm⁻¹). Similarly, the two C-C stretching modes above 1200 cm⁻¹ are also found in the same order in each neutral molecule.

In the radical anions, the C=O symmetric stretch consistently shifts downward by about 80 cm⁻¹ with respect to the neutral mode to about 1670 cm⁻¹, and is always the highest non-C-H stretching mode. The relative positions of the

C=O antisymmetric stretch and the two C=C stretches change slightly depending on the substitution pattern, and are all found between 1520 cm^{-1} and 1575 cm^{-1} , which is always higher than the two C-C stretches, which shift to $\sim 1450\text{ cm}^{-1}$ and $\sim 1230\text{ cm}^{-1}$, respectively. The mode assignments for the C=C stretching modes in $2\text{Q}^{\bullet-}$ are ambiguous due to mixing: ViPA results support the assignments shown in Table 3-3, but only by a difference of about 1% from each other.

Certain trends in the changes in the frequencies of vibrational modes through the series are evident. For instance, the C=O stretches of both the neutral and radical anion tend to shift to slightly lower frequencies as methyl substitution increases, which is consistent with the lengthening of the C=O bond shown in Table 3-2. C=C stretches tend to shift to slightly higher frequencies as substitution increases, as does the first C-C stretching mode. No clear trend is evident in the second C-C stretching mode through the series of methyl-substituted quinones.

For the important structural features and vibrational modes discussed above, PBQ and its radical anion provide reasonably good models of the rest of the methyl-substituted series. Apart from a small amount of vibrational shift and reordering (which in general follow predictable trends) dependent on the substitution level, similar vibrational modes occur at similar frequencies in PBQ and its radical anion as they do for the rest of the series. The bond lengths and bond angles of PBQ are clearly very similar to the rest of the methyl-substituted series, as are the changes upon one-electron gas-phase reduction.

3.2.3 Multiply Reduced and Protonated *para*-Quinones

3.2.3.1 Geometries

The current understanding of the function of the bacterial photosynthetic reaction center indicates that the secondary quinone first accepts two electrons, one at a time, and is then doubly protonated and leaves the reaction center to be replaced by a new quinone to restart the cycle. This process involves the formation of the radical anion, dianionic, protonated anion, and the doubly protonated hydroquinone. Some evidence also exists that the radical anion may be protonated to form a protonated neutral radical before the second electron is transferred.^{8,91-94} Because all of these species are important to a complete understanding of bacterial photosynthesis, we now present the results of HF/DF structural and vibrational frequency analysis on each of the species mentioned above. We showed in Section 3.2.2 that PBQ provides a good model for methyl substituted quinones, and so we first investigate its reduced and protonated forms. Next, because we also found that asymmetrically substituted quinones may show slight changes in the ordering of spectroscopically important vibrational modes relative to those of PBQ, we investigate the reduced and protonated forms of TMQ, which was recently shown to provide an excellent model for plastoquinone,⁶⁴ a quinone important in plant photosystems. Additionally, we include a discussion of the reduced and protonated forms of NQ, the parent molecule of menaquinone, which is used as an electron acceptor in the *Rp. viridis* reaction center.

All common reduced and protonated forms of PBQ were recently investigated with the BP86/6-31G(d,p) method.⁷⁴ However, as we noted above, the DF BP86 method (with a smaller, 6-31G(d) basis set) gave worse agreement

with the gas-phase experimental structure of DQ than did the B3LYP/6-31G(d) method. Also, Nonella noted in his study that there may be a systematic error with the BP86 method that affects the prediction of fundamental vibrational frequencies. Therefore, we present in Table 3-4 the calculated geometries of PBQ²⁻, PBQH⁻, PBQH₂, and PBQH[•], and in Table 3-5 a complete listing of the vibrational frequencies and assignments for all four species.

Our calculated structure of PBQ²⁻ shows that C=O bonds lengthen by approximately 0.04 Å with respect to the radical anion (see Table 3-2), while C=C bonds shorten by about 0.03 Å. C-C bonds shorten by about 0.01 Å, and C-H bond lengths increase by 0.009 Å. These changes are consistent with the work of Nonella, but our absolute C=C and C-C bond lengths are about 0.01 Å shorter, while our C=O bond lengths are 0.007 Å shorter.

Going from PBQ²⁻ to PBQH⁻, we find that the C-O bond distance on the protonated carbonyl group increases by 0.108 Å to 1.409 Å, in exact agreement with the change predicted by Nonella. Both studies also predict that the non-protonated C=O bond length decreases by about 0.03 Å. The two C-C bond lengths proximal to the protonated oxygen are predicted in both studies to decrease by about 0.05 Å, while the distal C-C bond lengths each grow by about 0.01 Å. Upon gaining the second proton to form the hydroquinone, PBQH₂, both the BP86/6-31G(d,p) calculations of Nonella and our B3LYP/6-31G(d) calculations predict similar geometry changes, but as was found for the other species, the bond lengths predicted with the B3LYP method were consistently about 0.01 Å shorter. Other experimental⁹⁵ and computational²⁸ determinations of the geometry of PBQH₂ report C-O bond distances from 1.364 Å to 1.394 Å, C-C bond distances of 1.369 Å to 1.393 Å, and C=C (as found in the unprotonated quinone) bond

Table 3-4. Calculated Bond Distances (Å) and Bond Angles (Deg) of 1,4-Benzoquinone (PBQ) in Various Reduced and Protonated States Using the B3LYP/6-31G(d) Method. See Table 3-2 for the geometries of the neutral and radical anion.

	PBQ ²⁻	PBQH ⁻	PBQH ₂	PBQH [•]
C1=O	1.301	1.409	1.373	1.355
C4=O	1.301	1.270	1.373	1.256
C1-C2	1.440	1.398	1.399	1.413
C1-C6	1.440	1.398	1.397	1.415
C4-C3	1.440	1.449	1.397	1.453
C4-C5	1.440	1.450	1.399	1.454
C2-C3	1.406	1.394	1.394	1.376
C5-C6	1.406	1.393	1.394	1.372
C2-X	1.099	1.097	1.089	1.089
C3-X	1.099	1.090	1.085	1.086
C5-X	1.099	1.090	1.089	1.086
C6-X	1.099	1.090	1.085	1.086
O-H	-	0.968	0.969	0.972
O-C1-C2	124.4	122.5	123.0	122.3
O-C4-C3	124.4	123.5	117.6	121.7
C6-C1-C2	111.2	118.6	119.4	120.7
C5-C4-C3	111.2	112.9	119.4	116.4
C1-C2-C3	124.4	121.2	120.5	119.9
C1-C6-C5	124.4	120.7	120.1	119.8
C4-C3-C2	124.4	123.0	120.1	121.4
C4-C5-C6	124.4	123.6	120.5	121.7
C1-C2-X	116.3	119.1	119.9	119.4
C1-C6-X	116.3	118.6	119.2	118.2
C4-C3-X	116.3	117.0	119.2	117.1
C4-C5-X	116.3	116.7	119.9	117.0
C-O-H	-	105.7	108.7	109.5

Table 3-5. Calculated Vibrational Frequencies (cm⁻¹) of 1,4-Benzoquinone (PBQ) in Various Reduced and Protonated States Using the B3LYP/6-31G(d) Method. See Table 3-3 for the frequencies of the neutral and radical anion species.

	sym ^a	PBQ ²⁻	PBQH ⁻	PBQH ₂	PBQH [•]
OH str.	-	-	3747	3756	3736
OH str.	-	-	-	3755	-
CH str.	a _g	3052	3155	3217	3224
CH str.	b _{2u}	3038	3148	3215	3221
CH str.	b _{1u}	3009	3127	3169	3207
CH str.	b _{3g}	3005	3127	3171	3207
C=O str.	a _g	1657	1674	1689	1639
C=O str.	b _{1u}	1545	1598	1571	1530
CC str.	b _{3g}	1476	1588	1663	1566
C=C str.	b _{2u}	1420	1473	1506	1478
C=C str.	a _g	1388	1428	1313	1459
CCH bend	b _{1u}	1343	1428	1288	1327
CC str.	b _{3g}	1259	1356	1379	1276
COH bend	-	-	1195	1216	1196
COH bend	-	-	-	1202	-
CC str.	b _{2u}	1180	1240	1383	1276
CCH bend	a _g	1146	1168	1194	1173
CCH bend	b _{2u}	1057	1090	1125	1110
CCC bend	b _{1u}	974	995	1029	990
CH wag	a _u	860	870	923	944
CH wag	b _{2g}	858	907	915	971
ring breathe	a _g	830	835	869	828
CH wag	b _{3u}	820	829	826	854
CC str.	b _{1u}	792	760	766	780
CH wag	b _{1g}	782	773	802	785
ring chair	b _{2g}	726	691	702	725
CC str.	b _{3g}	651	647	659	632
ring boat	b _{3u}	526	500	524	517
CCO bend	b _{3g}	481	463	448	460
CCC bend	a _g	473	466	471	466
ring boat	a _u	432	431	426	386
CCO bend	b _{2u}	363	346	342	369
CO chair	b _{2g}	363	335	370	324
OH wag	-	-	156	314	434
OH wag	-	-	-	311	-
CO boat	b _{3u}	154	144	158	136

^a The symmetries listed are appropriate for the vibrational modes of PBQ²⁻, and are provided for comparison purposes.

distances of 1.375 Å to 1.401 Å. Our calculated geometries and those of others^{66,74} all fall within the range of these experimental values.

The protonated neutral radical, PBQH[•], which could be formed as an intermediate in the photosynthetic processes, is structurally analogous to the protonated anion, PBQH⁻. Mohandas and Umapathy⁷⁰ have studied it with a variety of computational methods and discussed the geometry and in-plane vibrational modes. Our calculations predict that both the protonated and unprotonated C-O bond distances in PBQH[•] are shorter (by 0.054 Å and 0.014 Å, respectively) than those in PBQH⁻. Similarly, the C=C bond distances are about 0.02 Å shorter in PBQH[•], but the C-C bond distances are longer in PBQH[•] by about 0.014 Å in the case of the proximal bonds, and 0.004 Å for the distal C-C bonds. These results are in agreement with the work of Nonella, but, again, the B3LYP bond lengths are consistently significantly shorter (by about 0.01 Å) than those from BP86 calculations. Also, calculations⁵⁰ using the B3LYP method and the larger 6-311G(d,p) basis set (rather than the 6-31G(d) used by us or the 6-31G(d,p) used by Nonella) give bond lengths for the neutral radical that agree with our heavy atom bond distances to within an average absolute magnitude of about 0.003 Å, whereas they differ from the geometries of Nonella by about 0.010 Å on average. The trend evident in all calculations is that further reduction and protonation of PBQ tends to bring the bond angles closer to 120°, or more benzenoid, which is not surprising and consistent with previous conclusions.²⁸

The geometries of the reduced and protonated forms of TMQ and NQ are provided in the Supplemental Tables S3-7 and S3-8. The geometry changes through the series of reduced and protonated TMQ were very similar to those seen in PBQ, except that nearly all absolute bond lengths were ~0.005 Å longer in the

TMQ derivatives. Similar trends were also predicted for NQ derivatives, with a few minor exceptions. In terms of absolute bond lengths, the C2=C3 bond in NQ²⁻ was predicted to be 1.410 Å, 0.03 Å shorter than the C=C bonds of PBQ²⁻, whereas most other analogous bond lengths were quite similar. The overall trend is toward a naphthalene-like structure with increasing reduction and protonation, just as in PBQ derivatives the trend is toward a more benzenoid form. Full discussions of our calculation of geometries and properties of NQ, TMQ, and their radical anions are provided in Chapter 2 and elsewhere.^{63,64}

3.2.3.2 Vibrational Frequencies

3.2.3.2.1 The Dianionic: PBQ²⁻

Information on the vibrational spectra of PBQ²⁻ is limited. We are aware of only three experimental studies which report only about a dozen specific frequencies and two ranges of absorption (1500-1450 cm⁻¹ and 1280-1240 cm⁻¹). Nonella reported ten calculated frequencies using the BP86 method and the 6-31G(d,p) and 6-31G++(d,p) basis sets. Table 3-5 contains a complete list of the fundamental vibrational frequencies and their assignments based on the assignments of the parent molecule PBQ. In the spectroscopically important region, we predict seven fundamental vibrational frequencies to occur above about 1200 cm⁻¹. The highest of these is a C=O symmetric stretching mode at 1657 cm⁻¹. (We assign this as a C=O stretch because ViPA matches it to the PBQ C=O symmetric stretch with a similarity of about 90%. Total energy distribution analysis shows that this mode is 33% C=C stretch and 18% C=O stretch in PBQ²⁻, but about 70% C=O stretch in PBQ. Despite the energy transfer between the two bond types, ViPA clearly shows that these two modes are very similar, and are

therefore “matched” here. The difference occurs in the phase of the coupling between the C=O and C=C stretching motions, and this phenomena has been addressed in Sections 3.2.2 and 2.2.4.2) Remembering that B3LYP/6-31G(d) absolute frequencies are overestimated by about 4%, we find that the scaled frequency of 1593 cm^{-1} agrees well with the experimentally observed modes^{42,44} at 1594 cm^{-1} and 1596 cm^{-1} . The next highest mode is the C=O antisymmetric stretch at a scaled value of 1485 cm^{-1} (absolute value 1545 cm^{-1}), which we would assign to the experimentally observed mode⁹⁶ at 1468 cm^{-1} . The next mode is a C-C stretching mode at a scaled value of 1419 cm^{-1} , followed by a C=C antisymmetric stretching mode at a scaled value of 1365 cm^{-1} . Neither of these modes were observed in experimental studies, and only the lower mode is predicted to be IR active. Both of the next two modes (at scaled values of 1291 cm^{-1} and 1210 cm^{-1}) are near the experimentally determined absorption range⁴² of $1280\text{--}1240\text{ cm}^{-1}$, and the observed Raman mode⁴⁴ at 1275 cm^{-1} , but only the higher frequency mode should be IR active. The BP86/6-31G(d,p) calculations of Nonella and the MP2/6-31+G(d,p) calculations of Zhao et al.⁴⁴ are not significantly different from those presented here with the smaller 6-31G(d) basis set. However, experimental data is too limited to draw conclusions about which method is best for predicting vibrational modes of PBQ²⁻ in the spectroscopically interesting region.

3.2.3.2.2 The Protonated Anion: PBQH⁻

The next species believed to be formed during photosynthetic electron transport is the protonated anion, PBQH⁻. The calculated modes shown in Table 3-5 predict the ordering of the vibrations in the range $1200\text{--}1700\text{ cm}^{-1}$ to remain

unchanged with respect to those of PBQ²⁻, except that a C-C-H bending mode has shifted upward in frequency to occur at the same position as the C=C symmetric stretching mode. All vibrational modes in this region tend to increase in frequency upon protonation, consistent with the formation of a more benzenoid structure. The vibrational frequencies predicted for PBQH⁻ by Nonella⁷⁴ are in reasonable agreement with those calculated here, except that where our calculations predict C=C and C-C-H bending to occur at the same frequency (1428 cm⁻¹), Nonella finds similar modes to be separated by about 100 cm⁻¹. Zhao et al.⁴⁴ experimentally observed vibrational modes at 1625 cm⁻¹, 1270 cm⁻¹, 1160 cm⁻¹, and 836 cm⁻¹. The first of these modes is most easily assigned to our calculated, scaled C=O symmetric stretching mode at 1609 cm⁻¹, while the second and third experimental frequencies are probably assignable to the calculated C-C stretch and C-O-H bend at scaled frequencies of 1304 cm⁻¹ and 1148 cm⁻¹, respectively. The experimental frequency around 840 cm⁻¹ is not clearly assignable to any calculated frequency, but may be the ring-breathing mode calculated here to appear at a scaled value of 803 cm⁻¹.

3.2.3.2.3 The Neutral Radical: PBQH[•]

An intermediate species which may form during photosynthetic processes is the quinone neutral radical, PBQH[•]. Our calculated frequencies shown in Table 3-5 predict almost the same ordering of frequencies in the 1700-1200 cm⁻¹ range as was found for PBQH⁻, except that the antisymmetric C=O stretch and nearby C-C stretching mode are reversed, consistent with the theoretical work of Nonella.⁷⁴ Our four highest non-C-H stretching modes at scaled values of 1576 cm⁻¹, 1506 cm⁻¹, 1471 cm⁻¹, and 1421 cm⁻¹, correspond to the experimentally determined

modes^{40,44} at 1613 cm⁻¹, 1532 cm⁻¹, 1511 cm⁻¹, and 1432 cm⁻¹, respectively. These values, while ordered correctly, are generally in worse absolute agreement with experiment (by about 10-20 cm⁻¹) than those determined by Nonella with the BP86/6-31G(d,p) method. We agree with Nonella, however, that the experimental mode identified at 1532 cm⁻¹ and previously assigned to a mix of COH bending and C-O stretching is more appropriately assigned to a C-C stretch.

3.2.3.2.4 Hydroquinone: PBQH₂

After the secondary quinone in photosynthetic reaction centers has received two electrons and one proton, a second proton is accepted to form the hydroquinone, which then leaves the RC. A definitive description of the entire set of vibrational modes is not yet available. Table 3-5 gives a complete list of the vibrational modes of PBQH₂ determined with the B3LYP/6-31G(d) method. The highest non-hydrogen stretching mode is calculated to occur at a scaled frequency of 1624 cm⁻¹, and is assignable to the mode experimentally observed⁹⁷ at 1618 cm⁻¹. This mode is analogous to the C=O symmetric stretching mode of PBQ (but is predominantly C=C stretching by TED analysis). The next highest vibrational mode is calculated to appear at a scaled frequency of 1599 cm⁻¹, in agreement with the C-C stretching mode identified experimentally⁹⁷ at 1604 cm⁻¹. Next is the b_u C=O stretching mode at a scaled frequency of 1510 cm⁻¹, assignable to the experimentally identified^{97,98} mode at 1512-1521 cm⁻¹. This mode is followed by a calculated, scaled C=C b_u stretching mode at 1448 cm⁻¹, which may be assigned to the experimentally determined^{97,98} mode at 1447 cm⁻¹ or 1455 cm⁻¹. This mode shows a better agreement with the former experimental frequency than do previously calculated vibrations at 1456 cm⁻¹ and 1472 cm⁻¹, but is about the same

as the 1449 cm^{-1} frequency determined at a lower level of theory (BP86/3-21G).⁷⁴ The next B3LYP/6-31G(d) frequency occurs at a scaled frequency of 1330 cm^{-1} , and is identified as a b_u C-C stretching mode, and may be easily matched to the experimental^{97,98} frequency at 1331-1332 cm^{-1} , giving an agreement significantly better than previous theoretical calculations. Overall, comparing our calculated scaled vibrational frequencies to the same experimental frequencies used by Nonella, we find that B3LYP/6-31G(d) frequencies scaled by 0.9614 differ on average by 22 cm^{-1} from experimental values.

3.2.3.2.5 Reduced and Protonated TMQ and NQ

We include as Supplemental Tables S3-9 and S3-10 a complete set of vibrational frequencies and assignments (based on the descriptions of the parent, neutral molecules) for the reduced and protonated species of both TMQ and NQ. TMQ serves as a model for plastoquinone, and gives a representative system to study the effects of methyl groups on the spectra of its reduced and protonated analogues. In all four cases shown in Table S3-9, it can be seen that H-C-H bending vibrations should heavily complicate the vibrational spectra in the range 1550-1500 cm^{-1} . In the dianionic, TMQ^{2-} , we find that while the C=O symmetric stretch 1639 cm^{-1} is not far from that found in PBQ^{2-} at 1657 cm^{-1} , the C=O antisymmetric stretch is lowered by 40 cm^{-1} relative to the same mode in PBQ^{2-} , to 1505 cm^{-1} . We also find the same effect in TMQH^- , TMQH_2 , and TMQH^+ , where the C=O symmetric stretch is 20-30 cm^{-1} lower than that found in the related PBQ species, while the C=O antisymmetric stretching mode in the TMQ derivatives is lowered much more: 40-90 cm^{-1} . The highest C-C stretching modes in TMQ^{2-} , TMQH^- , and TMQH_2 at 1473 cm^{-1} , 1611 cm^{-1} , and 1681 cm^{-1} ,

respectively, are found within about 20 cm^{-1} of the similar modes in PBQ species, but the same vibration in $\text{TMQH}^\bullet$, which occurs at 1484 cm^{-1} , is 82 cm^{-1} lower than the same mode in $\text{PBQH}^\bullet$. The C=C antisymmetric stretching mode in the TMQ species occurs at higher frequencies than in the related PBQ derivatives, with the largest difference being about 100 cm^{-1} in the neutral radical. The C=C symmetric stretching mode is always found at a lower frequency than the antisymmetric C=C stretching mode across the TMQ series, just as it is in the PBQ series, and it usually occurs within about 40 cm^{-1} of the same mode in PBQ derivatives, sometimes higher and sometimes lower in frequency.

The calculated vibrational spectra of various reduced and protonated forms of NQ are shown in Table S3-10. The C=O and C=C stretching modes of these molecules are complicated by the presence of an additional C-C stretching mode (at 1598 cm^{-1} in NQ^{2-}) centered on the fused ring, which in the cases of NQH_2 and $\text{NQH}^\bullet$ is actually predicted to be the highest non-C-H stretching mode. Other important differences in the spectra of NQ species include the prediction that the C=O antisymmetric stretching mode in NQH_2 is actually slightly higher (by 8 cm^{-1}) than the C=O symmetric stretching mode, and that the C=O antisymmetric mode of $\text{NQH}^\bullet$ is calculated to appear about 30 cm^{-1} higher in frequency than the same vibrational mode in $\text{PBQH}^\bullet$. The C2=C3 stretching mode behaves across the NQ series in a very similar manner to the C=C symmetric stretching mode in the PBQ series, occurring at about 1400 cm^{-1} in all cases except NQH_2 , where it is predicted to lower to about 1300 cm^{-1} .

3.2.4 The Effect of Hydrogen Bonding on Neutral and Reduced PBQ

In photosynthetic reaction centers, quinones are known to be within hydrogen-bonding distance of several amino acids. FTIR spectroscopy is often used to study the binding of these quinones both before and during electron transport. In Section 3.2.3, we provided a detailed discussion of the effects of multiple reduction and protonation on the structure and spectra of three representative quinones: PBQ, TMQ, and NQ. Now, we present the results of calculations designed to predict the effects of single or double hydrogen bonding on the vibrational spectra of PBQ, PBQ^{•-}, and PBQ²⁻. Because of its smaller size, and because PBQ has been shown above to provide a reasonable model of geometrical and spectroscopic properties of *para*-quinones in general, it is the only quinone used for this study. Water molecules were used as simple sources of hydrogen bonds in the calculations as others have done to predict spin properties,²⁸ and no symmetry constraints were imposed. Each calculation was allowed to optimize freely, and no negative frequencies were obtained, indicating that all geometries were in a local minimum at least. No attempt was made to search for other energy minima.

The effect of hydrogen bonding by one or two waters on the geometry of the three species of PBQ are predictably small. The geometries from each calculation are shown in detail in Table S3-11. A few simple trends are evident. First, the C=O bond length for each quinone oxygen involved in a hydrogen bond increases by 0.007-0.027 Å, while in the case of single hydrogen bonds, the C=O bond length on the opposite side of the molecule decreases very slightly by 0.001-0.006 Å. C=C bond lengths remain virtually unchanged across the series when compared to non-hydrogen bonded species, while C-C bond lengths nearest

hydrogen-bound carbonyl groups tend to shorten by about 0.008 Å. The distance between the quinone oxygen and the nearest water hydrogen decreases by about 0.2 Å with each additional electron.

A complete list of the calculated vibrational frequencies and modes of PBQ, PBQ⁻, and PBQ²⁻ hydrogen bonded to one or two water molecules is provided in Tables S3-12 through S3-14, and the important quinone vibrations in the region 1800-1200 cm⁻¹ are condensed in Table 3-6. The most obvious effect of hydrogen bonding on PBQ is to switch the ordering of the two C=O stretching vibrations relative to the gas phase, causing the symmetric C=O stretch to occur at 1758 cm⁻¹, 19 cm⁻¹ higher than the antisymmetric C=O stretch. The changes in the difference between the two C=O stretching modes from about 2 cm⁻¹ in the gas phase, to near 20 cm⁻¹ for single hydrogen bonding, and back to about 8 cm⁻¹ for double hydrogen bonding ought to be distinguishable in experimental studies, and should reveal cases of hydrogen bonding to one vs. both quinone oxygens. C=C stretching modes in PBQ are not greatly affected by H-bonding, while the two highest C-C stretching modes both increase slightly from 1406 cm⁻¹ and 1328 cm⁻¹ to 1410 cm⁻¹ and 1337 cm⁻¹, respectively.

In PBQ⁻, it is the order of the C=C stretching modes which are reversed by hydrogen bonding. The C=C antisymmetric stretching mode increases from 1520 cm⁻¹ in the gas phase to 1525 cm⁻¹ with a single hydrogen bond and to 1530 cm⁻¹ with two hydrogen bonds. At the same time, the C=C symmetric stretch decreases from 1527 cm⁻¹ in the gas phase to 1515 cm⁻¹ and 1510 cm⁻¹ with one and two hydrogen bonds, respectively. C=O stretching modes in PBQ⁻ are virtually unaffected by hydrogen bonding, changing by no more than 4 cm⁻¹ each, but moving in opposite

Table 3-6. Important Vibrational Frequencies (cm⁻¹) of 1,4-Benzoquinone in Various Reduced and Hydrogen-Bonded States Determined with the B3LYP/6-31G(d) Method.

Mode ^a	PBQ·H ₂ O	PBQ·2H ₂ O	PBQ ^{•-} ·H ₂ O	PBQ ^{•-} ·2H ₂ O	PBQ ^{2•-} ·H ₂ O	PBQ ^{2•-} ·2H ₂ O
C=O asym str.	1739	1734	1571	1568	1540	1530
C=O sym str.	1758	1746	1674	1678	1658	1656
C=C sym str.	1689	1686	1515	1510	1386	1357
C=C asym str.	1659	1660	1525	1530	1429	1437
C-C str.	1410	1410	1469	1476	1499	1519
C-C str.	1337	1344	1245	1262	1201	1226

^a The ordering of the vibrational modes presented is the same as that found for the neutral and radical anion in Table 3-3 for ease of comparison.

directions and further apart. The two C-C stretching modes in this region both increase by about 10 cm^{-1} with each additional hydrogen bond.

For PBQ^{2-} , hydrogen bonding does not cause the frequencies in the region described in Table 3-6 to change ordering. The C=O symmetric stretch is virtually unchanged in frequency from the gas phase, while the antisymmetric C=O stretch decreases by 5 cm^{-1} with a single hydrogen bond, and a further 10 cm^{-1} with the second hydrogen bond. The C=C symmetric stretching mode is only lowered by 2 cm^{-1} with one hydrogen bond, but drops by nearly 30 cm^{-1} to 1357 cm^{-1} with the creation of the second hydrogen bond. The highest C-C stretching mode increases by 8 cm^{-1} with each additional hydrogen bond, while the next highest C-C stretching mode at 1259 cm^{-1} in the gas phase first decreases by almost 60 cm^{-1} to 1201 cm^{-1} with a single hydrogen bond, and then increases by 25 cm^{-1} upon formation of the second hydrogen bond.

An additional vibration in the same range as the C=O and C=C stretching modes is a C-H bending mode, calculated to occur at 1397 cm^{-1} in PBQ in the gas phase, which increases to 1401 cm^{-1} and 1407 cm^{-1} with the addition of one and two hydrogen bonds, respectively. In the radical anion, this mode shifts from 1388 cm^{-1} in the gas phase, to 1281 cm^{-1} with one hydrogen bond, and to 1295 cm^{-1} with two hydrogen bonds. In PBQ^{2-} , this vibration drops 32 cm^{-1} to 1311 cm^{-1} when only one H-bond is present, and increases slightly to 1319 cm^{-1} with the formation of the second hydrogen bond.

When $\text{PBQ}^{\bullet-}$ accepts a proton to form the neutral radical, B3LYP/6-31G(d) calculations predict that the C=O and C=C stretching modes will all shift downward by 30 cm^{-1} or more, whereas hydrogen bonding to the radical anion has very little effect on these modes, actually resulting in small increases in frequency

in the symmetric C=O stretch and the antisymmetric C=C stretching mode. On the other hand, when PBQ²⁻ accepts one or two protons to form the protonated anion or hydroquinone, all four of these vibrational modes shift upward with the first proton, while the antisymmetric C=O stretch and the symmetric C=C stretch both shift downward with the second proton. Hydrogen bonding to PBQ²⁻ results in slight decreases in both C=O stretching frequencies and in the symmetric C=C stretch, but an increase of almost 20 cm⁻¹ in the frequency of the antisymmetric C=C stretching mode.

3.2.6 Comparison to Experimental Results in Solution and in Reaction Centers

The purpose of the calculations described above is to assist in the interpretation of experimental results where the spectra may be complicated, especially in photosynthetic reactions centers. In the preceding discussion, we related our calculations on reduced and protonated species to previous experimental results. In this section, we compare our calculations of the vibrational spectra of hydrogen bonded PBQ to previous experimental results in hydrogen bonding environments such as those found in solutions and in reaction centers where the differences in the spectra when hydrogen bonding may occur.

Bauscher et al.⁴¹ have identified two IR bands of PBQ in MeCN at 1671 cm⁻¹ and 1658 cm⁻¹ which they assigned to C=O stretches. Previously calculated^{37,39} scaled frequencies place these two modes at 1690 cm⁻¹ and 1688 cm⁻¹ in the gas phase, and we have predicted here that they should switch order and move farther apart to 1667 cm⁻¹ and 1679 cm⁻¹ upon double hydrogen bonding. Bauscher et al. identify the same modes in MeOH at 1672 cm⁻¹ and 1661 cm⁻¹, a slight increase in frequency for each mode, whereas our prediction is

that the frequencies would decrease with hydrogen bonding. Only the antisymmetric (b_{1u}) C=O stretching mode should be IR active if PBQ retains D_{2h} symmetry in solution, so it is possible that there are solvent interactions in MeCN which serve to break the symmetry of PBQ. We note that the two experimental vibrations are 11-13 cm^{-1} apart, whereas our calculations predict that a doubly hydrogen bound PBQ would show a separation of 12 cm^{-1} in the two C=O stretches. Bauscher et al. also identify a C=C stretching mode at 1592 cm^{-1} in MeCN and 1595 cm^{-1} in MeOH. This is consistent with our calculated scaled PBQ·2H₂O C=C antisymmetric stretching mode at 1596 cm^{-1} , predicted to have remained almost unchanged in frequency from the scaled gas phase value (1595 cm^{-1}).

Recently, Zhao et al.⁴⁴ reported experimental Raman spectra of the totally symmetric vibrational modes PBQ^{•-} and PBQ²⁻ in water and MeCN, which should provide an excellent standard by which to judge our calculated effects of hydrogen bonding. First, we calculate a C=O symmetric stretching mode at 1678 cm^{-1} for doubly hydrogen bound PBQ^{•-}, and Zhao et al. identify a strong vibration at 1620 cm^{-1} , which agrees well with our scaled (by a factor of 0.9614) frequency at 1613 cm^{-1} . Schuler et al. also identified a mode at 1620 cm^{-1} , and noted that in D₂O, this frequency shifted up by 1.5 cm^{-1} . We checked this result of isotopic substitution and found that our calculated, scaled mode at 1613 cm^{-1} does increase by 1 cm^{-1} with deuterated water. In MeCN, Zhao et al. found a doublet at 1609 cm^{-1} and 1639 cm^{-1} . If the observed mode at 1609 cm^{-1} in MeCN corresponds to the mode at 1620 cm^{-1} observed in water, then this increase in frequency with H-bonding is consistent with our calculations, but the higher frequency mode in the observed doublet remains unexplained. Next, Zhao et al. identified a very weak

mode at 1503 cm^{-1} in MeCN which shifts to 1512 cm^{-1} in water. This mode was not observed by Schuler et al.⁴³ and could correspond to the C=O antisymmetric stretching mode predicted at a scaled frequency of 1507 cm^{-1} in doubly-H-bonded PBQ^{•-}, but our calculations would predict this mode to go down in frequency as H-bonding increased, and in strict D_{2h} symmetry, this mode would not be Raman active. Both experimental studies identified a mode at about 1434 cm^{-1} in water, and Zhao et al. report a similar mode at 1452 cm^{-1} in MeCN. This mode most closely corresponds to our calculated C=C symmetric stretching mode, which we predicted to increase by 10 cm^{-1} on going from the isolated to the doubly hydrogen-bound state. The experimental mode at 1161 cm^{-1} in water would seem to correspond to our calculated scaled mode at 1135 cm^{-1} , which is predicted to shift downward by 20 cm^{-1} in a non-hydrogen bonding environment, and indeed, Zhao et al. identified a mode in MeCN at 1143 cm^{-1} , which is qualitatively consistent with our calculations. Zhao et al. also identify a very weak mode at 964 cm^{-1} for PBQ^{•-} in water which does not match any of our calculated modes, and this mode is not identified in MeCN, possibly because of a large solvent line at 919 cm^{-1} . Weak modes identified at 830 cm^{-1} and 484 cm^{-1} (481 cm^{-1} in the work of Schuler et al.) in water would correspond to our calculated scaled frequencies of 796 cm^{-1} and 459 cm^{-1} for PBQ^{•-}·2H₂O, which are both predicted to have increased by 5 cm^{-1} and 10 cm^{-1} , respectively, relative to gas phase calculations. This is reasonably consistent with experimentally identified increases of 11 cm^{-1} and about 12 cm^{-1} . Zhao et al. also identify IR active vibrations of PBQ^{•-} at 1506 cm^{-1} , 1347 cm^{-1} , and 780 cm^{-1} in MeCN, which would most closely correspond to a calculated C=O antisymmetric stretching vibration at a scaled value of 1513 cm^{-1} , a C-C stretch and C-H bend at a scaled frequency of 1334 cm^{-1} , and a C-C

stretch at 791 cm^{-1} . These modes have not been identified in water, and so no comparison of the predicted changes upon hydrogen bonding is possible.

Only a limited amount of experimental data is available for PBQ^{2-} . Bauscher and Mäntele⁴¹ identified an IR peak at 1468 cm^{-1} in MeCN, which shifted to 1492 cm^{-1} in MeOH. They assigned it to a C-C stretching mode, and it most closely corresponds to our calculated mode at scaled values of 1419 cm^{-1} for PBQ^{2-} , 1441 cm^{-1} with one hydrogen bond, and 1460 cm^{-1} with two hydrogen bonds. Although the absolute agreement is less than perfect, we do predict a 40 cm^{-1} increase in frequency on hydrogen bonding, which is not inconsistent with the experimental increase of 24 cm^{-1} on going from MeCN to MeOH. Clark and Evans⁴² identified an IR mode at 1571 cm^{-1} for PBQ^{2-} which is not consistent with our calculations. But this mode could be consistent with the formation of PBQH^+ , supported by our calculated scaled frequency of this molecule at 1576 cm^{-1} , but a previous experimental study of PBQH^+ reported this frequency to occur at about 1615 cm^{-1} . Zhao et al. identified a Raman mode at 1596 cm^{-1} in MeCN, which matches well with our calculated scaled C=O symmetric stretching mode at 1593 cm^{-1} , but the experimental study reports that this mode increases in frequency by 25 cm^{-1} in water, whereas our calculations predict it to be virtually unchanged on hydrogen bonding. We performed ^{18}O isotopic substitution calculations and predicted this mode to decrease slightly by $1\text{--}4\text{ cm}^{-1}$, which is consistent with the experimental work, and we note that the peak at 1596 cm^{-1} in MeCN is extremely weak relative to the reported background noise. Therefore, either the location of this peak was incorrectly described by Zhao et al., or our calculations produced a rather large error given the reasonable successes in reproducing trends described earlier. Zhao et al. also report a Raman mode at

1275 cm^{-1} in MeCN which lowers by 13 cm^{-1} to 1262 cm^{-1} in water. This is consistent with our calculated scaled C=C symmetric stretching mode at 1305 cm^{-1} in doubly hydrogen bound PBQ²⁻, which is about 30 cm^{-1} lower than that predicted in the gas phase calculations.

In as far as PBQ may be used as a model for other quinones, especially those commonly found in bacterial RCs (ubiquinone and menaquinone), we can identify certain trends which may help to interpret the vibrational spectra of para-quinones *in vivo*. First, the C=O stretching frequencies tend downward and toward a greater separation as hydrogen bonding increases in neutral and dianionic species, though the downward trend is more marked in the neutral species. In the radical anions, the C=O symmetric stretch (the highest non-C-H stretching mode) is relatively unaffected by hydrogen bonding and even tends slightly upward (by about 4 cm^{-1}), while the next highest frequency, described here as a C=O antisymmetric stretch, tends steadily downward (by about 6 cm^{-1}) with increasing H-bonding. The C=C symmetric stretching mode also tends downward in all three states, with the most dramatic drop in the dianion of about 30 cm^{-1} . The C=C symmetric stretching mode is nearly unaffected by H-bonding in the neutral species, while in the radical anion and dianionic it tends to move higher in frequency with increasing hydrogen bonding by 10-17 cm^{-1} . The question then becomes whether or not these trends are or can be observed in experimental studies of reaction centers.

Breton et al. have done many FTIR studies on the binding of the primary and secondary acceptor quinones in *Rp. viridis* and *Rb. sphaeroides*.¹³ They have shown that the C=O stretching modes of neutral menaquinone (as Q_A) decrease from around 1660 cm^{-1} in CH₂Cl₂ solution to 1651 cm^{-1} and 1640 cm^{-1} in *Rb*.

sphaeroides, and to 1653 cm^{-1} and 1636 cm^{-1} in *Rp. viridis*. This seems to clearly indicate hydrogen bonding in each reaction center, though the differences in frequency lowering between the reaction centers may indicate different numbers or arrangements of hydrogen bonds. C=C stretching modes of menaquinone were shown to lower by about 10 cm^{-1} by Breton et al., which is reasonably consistent with our calculated lowering of about 7 cm^{-1} for the symmetric C=C stretching mode of PBQ upon H-bonding, but not with our calculated rise in frequency of 1 cm^{-1} for the antisymmetric C=C stretch. This could be reasonably explained by the obvious differences in structure between the two molecules.

Neutral ubiquinone has been shown by Breton et al. to have C=O stretching frequencies at 1663 cm^{-1} and 1650 cm^{-1} in solution which then drop by around 20 cm^{-1} in reaction centers at both the Q_A and Q_B positions. A C=C stretching mode was identified in solution at 1611 cm^{-1} which increases by 4-17 cm^{-1} in reaction centers, with the largest increase at the Q_A position. This may be consistent with the C=C stretching mode which was calculated to be nearly unaffected (increasing by 1 cm^{-1}) in H-bonded PBQ. There is much less data in the current literature suitable for the comparative effect of hydrogen bonding on quinone radical anions in reaction centers, and none that we are aware of for dianions.

3.3 Conclusions

Understanding the behavior of quinones as electron acceptors in bacterial photosynthetic reactions centers is an important part of understanding the overall process of photosynthesis. One way in which the function of these quinones can

be studied, both in vivo and in vitro, is by vibrational spectroscopy. The spectra of reduced, protonated, or hydrogen-bound quinones can be very complex, especially in reaction centers. Therefore, we have provided the first complete vibrational analysis of all six methyl-substituted derivatives of PBQ and their radical anions. We also report the first full theoretical descriptions of the vibrational spectra of reduced and protonated forms of three quinones (PBQ, TMQ, and NQ) which may serve as models of all native quinones involved in photosynthesis. We have identified specific trends that may be used to help interpretation of experimental vibrational studies of the intermediate stages of photosynthesis. In general, we had very good agreement with experimental vibrations, where available, and with previous theoretical studies. In fact, in most cases, B3LYP/6-31G(d) geometries and frequencies were at least as good as other calculations with larger, more computationally expensive methods and basis sets. One possible exception to this was with the neutral radical (e.g. PBQH[•]), where the agreement with previous frequencies and mode assignments was uncertain.

In addition to reduced and protonated quinones, we have also provided the first complete analysis of the effect of hydrogen bonding on a typical *para*-quinone, PBQ, in the neutral, radical anionic, and dianionic forms. Each of these species represents states believed to be formed during photosynthesis. In reaction centers, quinones are bound in reasonably specific sites where hydrogen bonding is possible with a number of nearby amino acid residues. The effects of hydrogen bonding on the vibrational spectra must be well understood in order to make definitive experimental assignments, which give insight into the specific processes involved in electron transfer. We used one or two water molecules to provide hydrogen bonds to each of the three PBQ species, and compared our results to

previous computed and experimentally measured numbers. We found that agreement was generally very good, with a few exceptions, when predicting trends of spectroscopically important vibrations, especially considering that only two “solvent” molecules were used in the calculations.

In general, the most easily observed vibrational mode which seems to show a consistent trend is the antisymmetric C=O stretch. This mode is predicted to decrease steadily upon reduction in the isolated molecule, but increases as protons are added to the reduced species or as more hydrogen bonds are formed. This trend is not followed by the neutral radical, whose spectra is difficult to predict. However, given the fact that only a relatively small amount of reliably assigned experimental vibrational modes is available for comparison, it is not yet possible to make definitive statements about the ability of the B3LYP/6-31G(d) method to predict the effects of hydrogen bonding on quinone spectra. Future experimental studies will undoubtedly clarify mode assignments, and it is our hope that the information provided in this study will assist that process.

3.4 Bibliography

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Table S3-1. Calculated Vibrational Frequencies (cm⁻¹) for 2-Methyl-1,4-benzoquinone (2Q) and the Corresponding Modes of the Radical Anion Determined with the B3LYP/6-31G(d) Method.

Mode		2Q	2Q ⁻	Mode		2Q	2Q ⁻
CH str.	a'	3219	3161	CH wag	a'	1028	1020
CH str.	a'	3201	3136	CCH bend	a''	1020	942
CH str.	a'	3197	3135	CH wag	a''	930	884
CH str.	a'	3144	3092	CC str.	a'	893	923
CH str.	a''	3113	3069	CH wag	a''	828	817
CH str.	a'	3057	3021	ring chair	a''	792	753
C=O str.	a'	1753	1674	CC str.	a'	767	797
C=O str.	a'	1752	1570	CC str.	a'	685	719
C=C str.	a'	1705	1557	ring boat	a''	590	598
C=C str.	a'	1669	1521	CCO bend	a'	582	591
HCH bend	a'	1507	1511	CCC bend	a'	485	505
HCH bend	a''	1499	1500	CCC bend	a'	440	455
HCH bend	a'	1443	1424	CCO bend	a'	400	383
CCH bend	a'	1404	1454	ring boat	a''	394	427
CCH bend	a'	1376	1371	CCMe	a'	289	285
CC str.	a'	1310	1224	CO chair	a''	268	341
CCH bend	a'	1242	1258	Me wag	a''	185	203
CMe str.	a'	1157	1158	Me torsion	a''	133	116
CCH bend	a'	1115	1103	CO boat	a''	91	131
CCH bend	a''	1075	1067				

Table S3-2. Calculated Vibrational Frequencies (cm⁻¹) for 2,3-Dimethyl-1,4-benzoquinone (23Q) and the Corresponding Modes of the Radical Anion Determined with the B3LYP/6-31G(d) Method.

Mode		23Q	23Q ⁻	Mode		23Q	23Q ⁻
CH str.	a ₁	3221	3164	CCH bend	a ₂	1078	1078
CH str.	b ₂	3203	3139	C-Me str.	a ₁	1077	1064
CH str.	b ₂	3167	3132	CCH bend	b ₁	1042	1046
CH str.	a ₁	3166	3131	CCH bend	a ₂	1017	940
CH str.	a ₂	3096	3035	CCH bend	a ₁	980	980
CH str.	b ₁	3089	3032	CCH bend	b ₁	856	830
CH str.	b ₂	3047	2999	CC str.	b ₂	823	846
CH str.	a ₁	3044	3001	ring chair	a ₂	792	760
C=O str.	a ₁	1742	1663	CCC bend	b ₂	762	773
C=O str.	b ₂	1742	1563	ring breathe	a ₁	629	650
C=C str.	a ₁	1701	1519	ring boat	b ₁	603	616
C=C str.	a ₁	1662	1552	CCO bend	b ₂	598	602
HCH bend	b ₁	1534	1541	ring boat	a ₂	510	507
HCH bend	a ₂	1520	1526	CCC bend	a ₁	468	481
HCH bend	b ₂	1510	1514	CCC bend	b ₂	444	469
HCH bend	a ₁	1507	1508	CCO bend	a ₁	414	400
CCH bend	a ₁	1437	1407	CCMe bend	b ₂	333	333
CCH bend	b ₂	1431	1402	CCMe bend	a ₁	305	304
CC str.	b ₂	1402	1449	Me wag	a ₂	269	347
CC str.	b ₂	1328	1355	Me wag	b ₁	255	250
CC str.	a ₁	1324	1235	Me torsion	b ₁	118	148
CCH bend	a ₁	1158	1158	CO chair	a ₂	116	138
C-Me str.	b ₂	1154	1178	Me torsion	a ₂	90	103
CCH bend	b ₂	1135	1122	CO boat	b ₁	88	127

Table S3-3. Calculated Vibrational Frequencies (cm⁻¹) for 2,5-Dimethyl-1,4-benzoquinone (25Q) and the Corresponding Modes of the Radical Anion Determined with the B3LYP/6-31G(d) Method.

Mode		25Q	25Q ^{-•}	Mode		25Q	25Q ^{-•}
CH str.	a _g	3199	3139	CCH bend	a _u	1075	1070
CH str.	b _u	3197	3136	CCH bend	b _g	1070	1064
CH str.	a _g	3146	3092	CCH bend	a _g	1035	1030
CH str.	b _u	3145	3092	CCH bend	b _u	1025	1016
CH str.	a _u	3112	3065	CH wag	a _u	935	889
CH str.	b _g	3112	3065	CH wag	b _g	883	866
CH str.	a _g	3058	3021	CC str.	b _u	806	838
CH str.	b _u	3057	3020	ring chair	b _g	787	749
C=O sym str.	a _g	1748	1677	CC str.	b _u	710	712
C=O asym str.	b _u	1745	1562	ring boat	a _u	674	670
C=C sym str.	a _g	1712	1544	ring breathe	a _g	671	715
C=C asym str.	b _u	1682	1571	CCO bend	a _g	615	617
HCH bend	b _u	1507	1507	CCC bend	a _g	494	509
HCH bend	a _g	1507	1514	CCO bend	b _u	403	383
HCH bend	a _u	1498	1500	CCC bend	a _g	401	422
HCH bend	b _g	1498	1500	ring boat	a _u	399	434
CCH bend	a _g	1443	1426	Me wag	b _g	365	379
CCH bend	b _u	1441	1424	CCMe bend	a _g	294	288
CC str.	a _g	1388	1457	CCMe bend	b _u	279	279
CCH bend	b _u	1377	1347	CO chair	b _g	197	247
CC str.	b _u	1273	1217	Me wag	a _u	161	172
CCH bend	a _g	1246	1246	Me torsion	a _u	131	111
CCC bend	b _u	1180	1166	Me torsion	b _g	129	111
CCH bend	a _g	1108	1127	CO boat	a _u	76	104

Table S3-4. Calculated Vibrational Frequencies (cm⁻¹) for 2,6-Dimethyl-1,4-benzoquinone (26Q) and the Corresponding Modes of the Radical Anion Determined with the B3LYP/6-31G(d) Method.

Mode		26Q	26Q ^{-•}	Mode		26Q	26Q ^{-•}
CH str.	a ₁	3199	3140	CCH bend	a ₂	1072	1067
CH str.	b ₂	3198	3136	CCH bend	b ₂	1062	1045
CH str.	a ₁	3145	3093	CCH bend	a ₁	1047	1043
CH str.	b ₂	3145	3092	CCC bend	b ₂	951	960
CH str.	b ₁	3112	3067	CH wag	b ₁	940	885
CH str.	a ₂	3112	3066	CCC bend	a ₁	912	945
CH str.	a ₁	3058	3021	CH wag	a ₂	875	856
CH str.	b ₂	3057	3020	ring chair	b ₁	795	760
C=O sym str.	a ₁	1749	1675	CC str.	a ₁	769	799
C=O asym str.	a ₁	1743	1566	ring boat	b ₁	657	646
C=C sym str.	a ₁	1712	1542	CCC bend	b ₂	616	622
C=C asym str.	b ₂	1681	1567	ring breathe	a ₁	570	582
HCH bend	a ₁	1508	1511	CCO bend	b ₂	468	494
HCH bend	b ₂	1506	1513	ring boat	a ₂	451	482
HCH bend	b ₁	1498	1499	CCC bend	a ₁	447	464
HCH bend	a ₂	1497	1499	CCO bend	b ₂	397	380
HCH bend	b ₂	1442	1423	CO chair	b ₁	312	355
CCH bend	a ₁	1442	1424	CCMe	b ₂	305	297
CC str.	b ₂	1395	1448	CCMe	a ₁	269	270
CCH bend	a ₁	1345	1353	Me wag	a ₂	208	230
CC str.	b ₂	1312	1261	Me wag	b ₁	164	180
CCH bend	b ₂	1225	1208	Me torsion	b ₁	128	104
CMe str.	a ₁	1204	1198	Me torsion	a ₂	126	101
CCH bend	b ₁	1077	1068	CO boat	b ₁	78	116

Table S3-5. Calculated Vibrational Frequencies (cm⁻¹) for 2,3,5-Trimethyl-1,4-benzoquinone (TMQ) and the Corresponding Modes of the Radical Anion Determined with the B3LYP/6-31G(d) Method.

Mode		TMQ	TMQ ⁻	Mode		TMQ	TMQ ⁻
C-H str.	a'	3198	3142	CCH bend	a''	1078	1079
C-H str.	a'	3167	3134	CCH bend	a''	1074	1067
C-H str.	a'	3164	3132	CCH bend	a'	1056	1044
C-H str.	a'	3143	3093	CCH bend	a''	1040	1046
C-H str.	a''	3115	3068	CCH bend	a'	1018	1015
C-H str.	a''	3096	3035	CMe str.	a'	939	946
C-H str.	a''	3088	3033	CCH bend	a''	909	876
C-H str.	a'	3058	3022	CC str.	a'	829	854
C-H str.	a'	3046	2999	ring chair	a''	789	759
C-H str.	a'	3043	3002	CCC bend	a'	704	714
C=O sym. str.	a'	1741	1668	ring boat	a''	688	680
C=O asym. str.	a'	1736	1556	CCO bend	a'	648	649
C=C sym. str.	a'	1714	1541	CC str.	a'	563	576
C=C asym. str.	a'	1673	1562	ring torsion	a''	513	518
HCH bend	a''	1533	1541	CCC bend	a'	471	487
HCH bend	a''	1519	1527	CCO bend	a'	421	401
HCH bend	a'	1509	1516	CCC bend	a'	411	436
HCH bend	a'	1507	1511	CMe wag	a''	372	390
HCH bend	a'	1507	1509	Me rock	a'	341	339
HCH bend	a''	1499	1500	Me rock	a'	306	307
CCH bend	a'	1442	1424	Me rock	a'	279	279
CCH bend	a'	1433	1409	Me wag	a''	259	281
CCH bend	a'	1428	1398	CO chair	a''	173	186
CC str.	a'	1382	1451	Me torsion	a''	138	119
CC str.	a'	1332	1335	Me torsion	a''	115	156
CC str.	a'	1284	1230	Me wag	a''	114	140
CCC bend	a'	1215	1213	CO boat	a''	75	102
CCH bend	a'	1136	1123	Me torsion	a''	60	102
CMe str.	a'	1121	1140				

Table S3-6. Calculated Vibrational Frequencies (cm⁻¹) for 2,3,5,6-Tetramethyl-1,4-benzoquinone (DQ) and the Corresponding Modes of the Radical Anion Determined with the B3LYP/6-31G(d) Method.

Mode		DQ	DQ ⁻	Mode		DQ	DQ ⁻
CH str.	b _{2u}	3171	3139	CCH bend	b _{1g}	1121	1112
CH str.	b _{1g}	3170	3138	CCH bend	b _{3g}	1080	1077
CH str.	a _g	3170	3139	CCH bend	a _u	1077	1083
CH str.	b _{3u}	3169	3137	CCH bend	b _{3u}	1055	1032
CH str.	b _{3g}	3094	3036	CCH bend	b _{1u}	1051	1050
CH str.	a _u	3094	3036	CCH bend	b _{2g}	1032	1049
CH str.	b _{1u}	3088	3034	CCH bend	a _g	1004	1003
CH str.	b _{2g}	3087	3033	CMe str.	b _{1g}	1001	1027
CH str.	b _{2u}	3046	3000	CMe str.	b _{3u}	894	887
CH str.	b _{1g}	3046	3000	CC str.	b _{2u}	841	861
CH str.	a _g	3044	3004	ring chair	b _{3g}	787	763
CH str.	b _{3u}	3043	3001	ring boat	b _{1u}	718	707
C=O str.	b _{2u}	1727	1546	CCO bend	b _{1g}	685	680
C=O str.	a _g	1725	1654	CCC bend	b _{2u}	621	617
C=C str.	a _g	1708	1654	ring boat	a _u	553	557
C=C str.	b _{3u}	1673	1556	ring breathe	a _g	546	560
HCH bend	b _{1u}	1536	1543	CCC bend	a _g	443	462
HCH bend	b _{2g}	1535	1543	CCO bend	b _{3u}	439	425
HCH bend	b _{3g}	1521	1527	Me wag	b _{3g}	436	429
HCH bend	a _u	1520	1528	CCC bend	b _{1g}	420	435
HCH bend	b _{1g}	1511	1521	CCMe bend	b _{1g}	364	368
HCH bend	b _{2u}	1508	1512	Me wag	b _{2g}	322	330
HCH bend	a _g	1508	1509	CCMe bend	b _{3u}	314	312
HCH bend	b _{3u}	1506	1508	CCMe bend	a _g	303	304
CCH bend	a _g	1436	1411	CCMe bend	b _{2u}	301	304
CCH bend	b _{3u}	1435	1412	Me wag	b _{1u}	206	208
CCH bend	b _{1g}	1431	1393	CO chair	b _{3g}	117	157
CCH bend	b _{2u}	1428	1400	Me torsion	b _{1u}	115	150
CC str.	b _{1g}	1352	1450	Me torsion	b _{2g}	110	147
CC str.	b _{3u}	1314	1233	Me wag	a _u	109	125
CMe str.	b _{2u}	1281	1313	Me trosion	b _{3g}	87	116
CMe str.	a _g	1158	1174	Me torsion	a _u	77	109
CCH bend	b _{2u}	1151	1136	CO boat	b _{1u}	65	96

Table S3-7. Calculated Bond Distances (Å) and Bond Angles (Deg) of 2,3,5-Trimethyl-1,4-benzoquinone (TMQ) in Various Reduced and Protonated States Using the B3LYP/6-31G(d) Method. See Table 3-1 for the geometries of the neutral and radical anion.

	TMQ ²⁻	TMQH ⁻	TMQH ₂	TMQH [•]
C1=O	1.303	1.410	1.378	1.410
C4=O	1.306	1.278	1.380	1.278
C1-C2	1.447	1.402	1.404	1.402
C1-C6	1.433	1.397	1.394	1.397
C4-C3	1.441	1.451	1.404	1.451
C4-C5	1.437	1.446	1.402	1.446
C2-C3	1.415	1.404	1.407	1.404
C5-C6	1.403	1.392	1.396	1.392
C2-X	1.512	1.514	1.510	1.514
C3-X	1.513	1.509	1.510	1.509
C5-X	1.510	1.505	1.511	1.505
C6-X	1.097	1.096	1.090	1.096
O1-H	-	0.969	1.969	0.969
O4-H	-	-	0.968	-
O-C1-C2	123.8	119.2	117.8	119.2
O-C4-C3	124.3	123.4	117.2	123.4
C6-C1-C2	113.0	119.8	120.7	119.8
C5-C4-C3	113.9	115.3	121.4	115.3
C1-C2-C3	122.9	119.5	118.9	119.5
C1-C6-C5	124.6	121.8	121.2	121.8
C4-C3-C2	123.2	122.5	119.7	122.5
C4-C5-C6	122.5	121.1	118.2	121.1
C1-C2-X	115.8	119.8	120.3	119.8
C1-C6-X	116.2	118.9	119.6	118.9
C4-C3-X	115.7	116.3	120.0	116.3
C4-C5-X	116.1	116.8	120.7	116.8
C1-O-H	-	105.5	108.3	105.5
C4-O-H	-	-	108.9	-

Table S3-8. Calculated Bond Distances (Å) and Bond Angles (deg) of 1,4-Naphthoquinone (NQ) in Various Reduced and Protonated Forms Determined with the B3LYP/6-31G(d) Method. See Tables 2-1 and 2-2 for the geometries of the neutral and radical anion species.

	NQ ²⁻	NQH ⁻	NQH ₂	NQH [•]
C1=O	1.294	1.407	1.373	1.357
C4=O	1.294	1.265	1.373	1.247
C2-C3	1.410	1.403	1.416	1.383
C9-C10	1.456	1.440	1.430	1.421
C6-C7	1.426	1.414	1.414	1.406
C1-C2	1.424	1.386	1.376	1.401
C3-C4	1.424	1.429	1.376	1.449
C1-C9	1.464	1.415	1.428	1.437
C4-C10	1.464	1.484	1.428	1.483
C8-C9	1.417	1.425	1.419	1.413
C5-C10	1.417	1.408	1.419	1.401
C5-C6	1.385	1.385	1.378	1.389
C7-C8	1.385	1.381	1.378	1.385
O1-H	-	0.968	0.969	09.70
O4-H	-	-	0.969	-
O-C1-C2	124.8	122.5	123.3	122.1
O-C4-C3	124.8	124.4	123.3	121.7
C2-C1-C9	112.3	119.6	120.3	121.3
C3-C4-C10	112.3	114.0	120.3	116.5
C1-C2-C3	124.9	122.6	120.6	121.0
C4-C3-C2	124.9	122.5	120.6	121.9
C1-C9-C8	119.3	122.9	121.9	122.0
C4-C10-C5	119.3	118.7	121.9	119.7
C1-C9-C10	122.8	118.7	119.1	118.5
C4-C10-C9	122.8	122.6	119.1	120.9
C7-C8-C9	122.7	121.1	120.6	120.0
C6-C5-C10	122.7	122.0	120.6	120.5
C5-C6-C7	119.4	119.3	120.4	120.2
C8-C7-C6	119.4	120.5	120.4	120.4
	-	106.0	108.5	109.4
	-	-	108.5	-

Table S3-9. Vibrational Frequencies (cm⁻¹) for 2,3,5-Trimethyl-1,4-benzoquinone (TMQ) in Various Reduced and Protonated Forms Determined with the B3LYP/6-31G(d) Method.

Mode	TMQ ²⁻	TMQH ⁻	TMQH ₂	TMQH ⁺	Mode	TMQ ²⁻	TMQH ⁻	TMQH ₂	TMQH ⁺
OH str.	-	-	3776	-	CCH bend	1108	1132	1126	1140
OH str.	-	3751	3758	3740	CCH bend	1071	1080	1084	1078
CH str.	3070	3115	3171	3168	CCH bend	1057	1065	1067	1062
CH str.	3066	3127	3166	3167	CCH bend	1047	1051	1056	1044
CH str.	3047	3066	3154	3146	CCH bend	1020	1037	1048	1058
CH str.	3016	3061	3126	3131	CCH bend	1005	1013	1026	1020
CH str.	2969	3041	3057	3107	CMe str.	941	955	951	952
CH str.	2950	2999	3015	3052	CC str.	856	851	855	858
CH str.	2923	2998	3082	3079	CCH bend	833	809	823	844
CH str.	2917	2972	3041	3036	ring chair	745	750	720	749
CH str.	2914	3040	3084	3094	CCC bend	715	721	707	714
CH str.	2905	3007	3040	3048	ring boat	680	627	644	646
C=O s. str.	1639	1653	1653	1637	CCC bend	649	629	616	633
HCH bend	1537	1540	1536	1534	ring breathe	575	567	580	571
HCH bend	1529	1520	1514	1527	ring boat	511	504	532	520
HCH bend	1523	1523	1520	1519	CCC bend	493	492	493	484
HCH bend	1523	1532	1541	1531	CCC bend	447	446	453	437
HCH bend	1515	1510	1522	1504	Me wag	393	372	401	382
C=O as. str.	1505	1562	1485	1484	CCO bend	376	370	368	390
HCH bend	1487	1492	1521	1498	CCO bend	330	319	332	334
CC str.	1473	1611	1681	1484	OH torsion	-	-	321	433
C=C as. str.	1446	1491	1545	1580	Me rock	296	306	320	314
C=C s. str.	1427	1400	1363	1447	Me wag	289	294	296	280
CCH bend	1397	1426	1442	1439	Me rock	273	275	292	281
CCH bend	1360	1400	1436	1422	OH torsion	-	164	235	-
CCH bend	1355	1430	1447	1418	CO chair	187	193	193	190

Table S3-9 Continued

COH bend	-	-	1378	1352	Me torsion	180	81	127	82
COH bend	-	1324	1306	-	Me torsion	148	134	140	131
CC str.	1307	1292	1282	1315	Me wag	142	143	55	132
CC str.	1203	1202	1216	1205	CO boat	116	118	114	100
CC str.	1198	1245	1253	1254	Me torsion	64	107	168	109
CMe str.	1130	1083	1111	1114					

Table S3-10. Vibrational Frequencies (cm⁻¹) for 1,4-Naphthoquinone (NQ) in Various Reduced and Protonated Forms Determined with the B3LYP/6-31G(d) Method.

Mode	NQ ²⁻	NQH ⁻	NQH ₂	NQH ⁺	Mode	NQ ²⁻	NQH ⁻	NQH ₂	NQH ⁺
OH str.	-	-	3758	-	ring breathe	1013	1048	1044	1046
OH str.	-	3754	3756	3740	CH wag	957	978	994	1006
CH str.	3150	3192	3229	3227	CH wag	934	935	965	977
CH str.	3147	3188	3227	3225	CH wag	869	867	858	923
CH str.	3091	3155	3200	3204	CH wag	818	813	798	826
CH str.	3075	3167	3181	3224	ring chair	815	769	785	772
CH str.	3062	3132	3184	3189	CCC bend	813	804	816	806
CH str.	3042	3075	3165	3160	CCC bend	761	763	756	767
C=O s. str.	1637	1677	1650	1618	CH wag	736	846	887	900
CC str.	1598	1619	1692	1657	CH wag	731	760	784	786
C=O as. str.	1565	1627	1658	1591	CCC bend	706	709	722	714
CC str.	1524	1567	1576	1572	ring chair	633	657	634	661
CCH bend	1477	1484	1502	1491	ring boat	629	588	624	608
CCH bend	1449	1465	1523	1539	CCO bend	604	588	571	589
CCH bend	1422	1547	1460	1474	CCC bend	560	553	558	554
C2=C3 str.	1402	1399	1300	1416	ring torsion	476	479	472	477
CC str.	1354	1373	1401	1374	CCC bend	474	479	490	472
COH bend	-	1317	1323	1352	CCC bend	466	459	464	457
COH bend	-	-	1249	-	ring boat	438	428	432	437
CCH bend	1316	1281	1284	1315	CO chair	380	360	392	340
CCH bend	1226	1240	1231	1263	CCO bend	345	340	335	355
CC str.	1221	1226	1445	1233	OH wag	-	-	332	408
CCH bend	1165	1173	1183	1171	OH wag	-	160	324	-
CCH bend	1140	1157	1176	1157	CCC bend	267	260	265	266
CCH bend	1118	1149	1188	1189	butterfly tors.	208	201	196	192
CCC bend	1078	1079	1080	1088	ring-ring twist	148	134	145	139
CCH bend	1040	1029	1066	1073	CO boat	126	123	137	115

Table S3-11. Calculated Bond Distances (Å) and Bond Angles (Deg) of 1,4-Benzoquinone (PBQ) in Various Reduced and Hydrogen Bonded States Using the B3LYP/6-31G(d) Method.

	PBQ ·H ₂ O	PBQ ·2H ₂ O	PBQ ^{•-} ·H ₂ O	PBQ ^{•-} ·2H ₂ O	PBQ ²⁻ ·H ₂ O	PBQ ²⁻ ·2H ₂ O
C1=O	1.232	1.231	1.278	1.276	1.328	1.319
C4=O	1.224	1.231	1.264	1.276	1.295	1.319
C1-C2	1.483	1.483	1.447	1.447	1.426	1.427
C1-C6	1.484	1.486	1.447	1.448	1.427	1.429
C4-C3	1.487	1.485	1.453	1.448	1.442	1.428
C4-C5	1.487	1.483	1.454	1.448	1.440	1.426
C2-C3	1.342	1.344	1.371	1.371	1.403	1.402
C5-C6	1.343	1.344	1.372	1.371	1.405	1.402
C2-X	1.086	1.086	1.090	1.089	1.096	1.095
C3-X	1.087	1.088	1.089	1.088	1.097	1.094
C5-X	1.086	1.086	1.089	1.088	1.096	1.095
C6-X	1.088	1.088	1.089	1.088	1.095	1.094
O1--H	1.982	1.990	1.763	1.777	1.559	1.583
O4--H	-	1.989	-	1.777	-	1.585
O-C1-C2	120.4	120.6	121.5	121.5	122.4	122.5
O-C4-C3	121.2	121.4	122.6	122.7	124.1	124.4
C6-C1-C2	118.0	118.1	115.6	115.8	113.0	113.0
C5-C4-C3	117.3	118.1	114.8	115.8	111.5	113.1
C1-C2-C3	121.0	121.3	122.3	122.4	123.7	123.7
C1-C6-C5	120.7	120.6	122.1	121.9	123.5	123.3
C4-C3-C2	121.3	120.6	122.5	121.8	124.1	123.2
C4-C5-C6	121.6	121.3	122.7	121.3	124.2	123.7
C1-C2-X	116.0	116.0	116.4	116.5	116.6	116.7
C1-C6-X	115.7	115.8	116.4	116.5	117.1	117.2
C4-C3-X	115.9	115.8	116.3	116.5	116.4	117.2
C4-C5-X	115.8	116.1	116.3	116.5	116.4	116.7
C1-O--H	113.7	113.7	115.0	114.8	113.5	114.0
C4-O--H	-	113.7	-	114.8	-	113.8

Table S3-12. Calculated Vibrational Frequencies (cm⁻¹) for 1,4-Benzoquinone (PBQ) Hydrogen Bonded to One or Two Waters.

Mode	PBQ ·H ₂ O	PBQ ·2H ₂ O	Mode	PBQ ·H ₂ O	PBQ ·2H ₂ O
OH str.	-	3806	ring chair	798	798
OH str.	3805	3805	ring breathe	780	782
OH str.	-	3644	CH wag	779	797
OH str.	3641	3642	CC str.	759	764
CH str.	3222	3217	HOH rot	626	628
CH str.	3215	3215	HOH rot	-	614
CH str.	3205	3190	CCC bend	605	609
CH str.	3190	3189	ring boat	527	538
C=O sym. str.	1758	1746	CCO bend	464	470
HOH bend	-	1736	CCC bend	456	458
C=O asym. str.	1739	1734	CCO bend	422	435
HOH bend	1736	1735	ring boat	344	349
C=C sym. str.	1689	1686	HOH rot	-	323
C=C asym. str.	1659	1660	HOH rot	319	318
CC str.	1410	1410	CO chair	244	216
CCH bend	1401	1407	HOH trans/tors	217	247
CC str.	1337	1344	HOH trans/tors	-	223
CCH bend	1250	1264	HOH trans/tors	157	163
CCH bend	1186	1194	HOH trans/tors	-	156
CCH bend	1100	1113	HOH trans/tors	99	106
CH wag	1061	1063	CO boat	98	97
CH wag	1021	1056	HOH trans/tors	-	84
CCC bend	953	955	HOH trans/tors	54	70
CH wag	919	926	HOH trans/tors	-	34

Table S3-13. Calculated Vibrational Frequencies (cm⁻¹) for 1,4-Benzoquinone Radical Anion (PBQ^{•-}) Hydrogen Bonded to One or Two Waters.

Mode	PBQ ^{•-} ·H ₂ O	PBQ ^{•-} ·2H ₂ O	Mode	PBQ ^{•-} ·H ₂ O	PBQ ^{•-} ·2H ₂ O
OH str.	-	3781	HOH rot	890	870
OH str.	3779	3781	CH wag	868	877
OH str.	3300	3343	ring breathe	826	828
OH str.	-	3333	CC str.	794	797
CH str.	3178	3187	CH wag	792	808
CH str.	3169	3184	ring chair	755	754
CH str.	3152	3163	CCC bend	636	640
CH str.	3146	3161	ring boat	532	541
HOH bend	-	1776	CCO bend	483	492
HOH bend	1777	1773	CCC bend	474	477
C=O sym. str.	1674	1678	HOH rot	-	438
C=O asym. str.	1571	1568	HOH rot	444	435
C=C asym. str.	1525	1530	CCO bend	411	434
C=C sym. str.	1515	1510	ring boat	399	402
CC str.	1469	1476	CO chair	331	333
CC str.	1388	1391	HOH trans/tors	211	225
CCH bend	1281	1295	HOH trans/tors	-	198
CC str.	1245	1262	HOH trans/tors	-	195
CCH bend	1171	1181	HOH trans/tors	185	190
CCH bend	1090	1104	CO boat	138	138
CH wag	981	987	HOH trans/tors	87	94
CCC bend	972	979	HOH trans/tors	-	75
CH wag	952	984	HOH trans/tors	48	63
HOH rot	-	878	HOH trans/tors	-	34

Table S3-14. Calculated Vibrational Frequencies (cm⁻¹) for 1,4-Benzoquinone Dianion (PBQ²⁻) Hydrogen Bonded to One or Two Waters.

Mode	PBQ ²⁻ ·H ₂ O	PBQ ²⁻ ·2H ₂ O	Mode	PBQ ²⁻ ·H ₂ O	PBQ ²⁻ ·2H ₂ O
OH str.	-	3758	CH wag	874	896
OH str.	3754	3758	ring breathe	836	845
CH str.	3089	3113	CH wag	826	834
CH str.	3071	3105	CC str.	795	794
CH str.	3048	3075	CH wag	789	805
CH str.	3038	3075	ring chair	726	727
OH str.	2500	2635	CCC bend	658	663
OH str.	-	2618	HOH rot	609	592
HOH bend	1815	1818	HOH rot	-	591
HOH bend	-	1811	ring boat	531	538
C=O sym. str.	1658	1656	CCO bend	497	511
C=O asym. str.	1540	1530	CCC bend	474	474
CC str.	1499	1519	ring boat	435	437
C=C asym. str.	1429	1437	CCO bend	397	427
C=C sym. str.	1386	1357	CO chair	367	375
CCH bend	1311	1319	HOH trans/tors	262	274
CC str.	1276	1293	HOH trans/tors	-	240
CC str.	1201	1226	HOH trans/tors	236	224
CCH bend	1158	1170	HOH trans/tors	-	222
CCH bend	1072	1090	CO boat	153	154
HOH rot.	-	1031	HOH trans/tors	-	102
HOH rot.	1037	1026	HOH trans/tors	93	78
CCC bend	984	995	HOH trans/tors	37	46
CH wag	883	894	HOH trans/tors	-	26

CHAPTER 4

Predicting the Electron Affinities and Aqueous One-electron Reduction Potentials of Quinones with Hybrid Hartree-Fock/Density Functional Theory and Molecular Dynamics Methods¹⁻⁵

4.1 Introduction

The formation of a radical through the gain or loss of a single electron is of fundamental importance to a wide range of disciplines. Besides the obvious field of electrochemistry,^{6,7} electron transfer (ET) processes are important in inorganic, organic, and biochemical reactions.⁸⁻¹⁰ ET is an essential step in photosynthesis, the basic means by which plants and some bacteria store and use light energy.¹¹⁻¹³ In all known photosynthetic processes, the basic molecule which serves as an electron acceptor is a *para*-quinone derivative. For instance, plastoquinone-9 (see Figure 4-1) is used in photosystem II,^{12,14-16} while in the *Rhodobacter sphaeroides* photosynthetic reaction center, it is ubiquinone-10 that is used as an electron acceptor.^{17,18} After reduction, these quinones are protonated and move out of the reaction center to help create a cross-membrane charge gradient which supplies the energy necessary to carry out other synthetic processes. Various quinone derivatives related to those mentioned above have been used as replacements in photosynthetic reaction centers in attempts to further understand the chemical processes involved.¹⁹⁻²⁴ In this study, we investigate the possibility of predicting the one-electron reduction potentials (RPs) and electron affinities (EAs) for a variety of *para*-quinones, which would make it

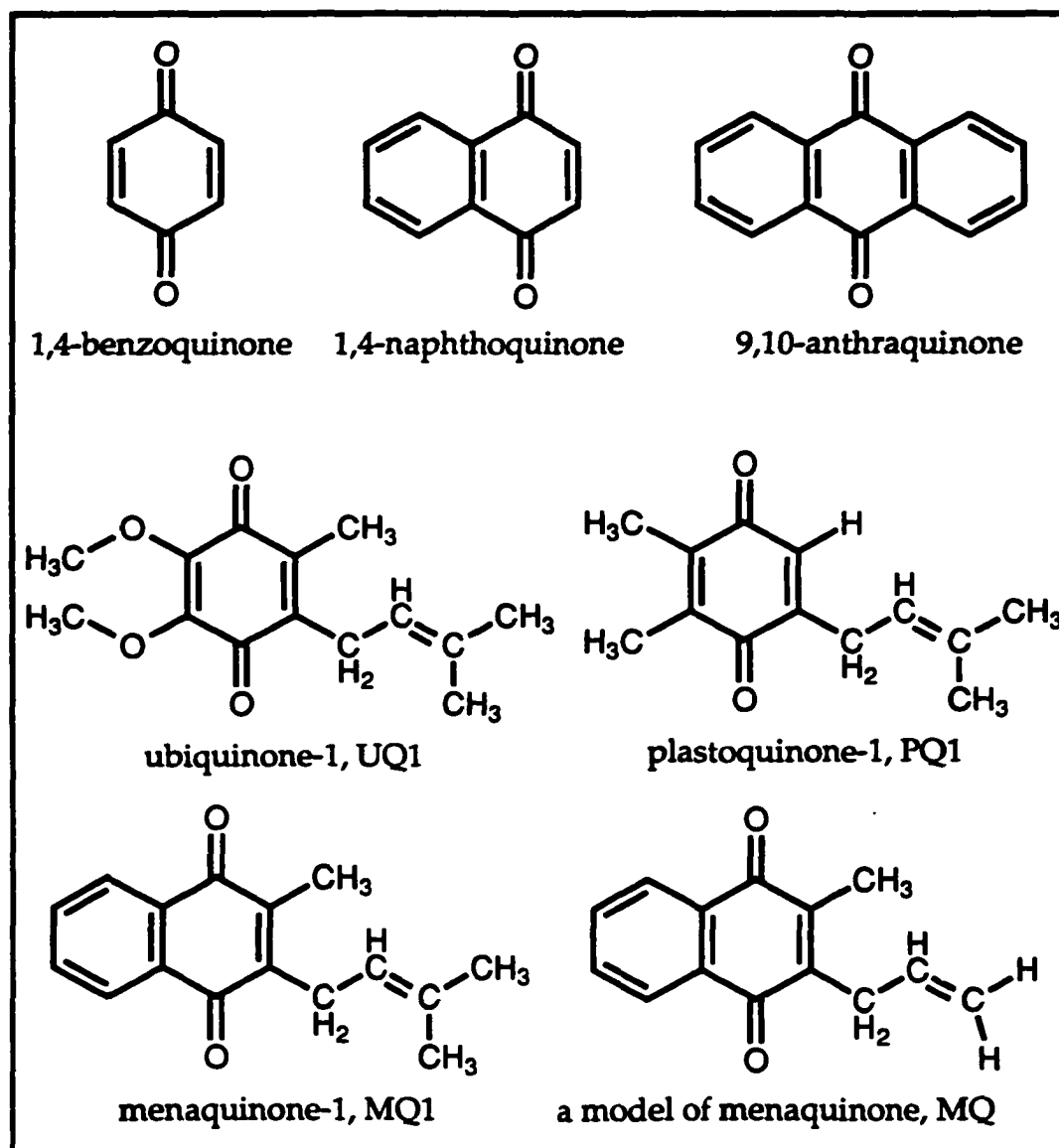


Figure 4-1. A Variety of Biologically Important Quinones and Related Compounds Used in this Study.

possible to perform a multitude of theoretical studies on photosynthetic processes that would help interpret experimental data and possibly suggest new experiments.

Calculating one-electron reduction potentials is a formidable task that requires accurate modeling of energy changes due to changes in non-bonded interactions (solvation) as well as changes in intramolecular bonding. Various techniques which account for both intra- and intermolecular energetic contributions are available, ranging from complex *ab initio* molecular dynamics methods²⁵⁻³² to simple methods involving a correlation in gas-phase electron affinities.³³⁻³⁵ Recently, a new method of calculating RPs was developed that allows for the separation of the contributions from intermolecular and intramolecular energy changes through the use of a thermodynamic cycle (Figure 4-2).^{36,37} This method has been shown to accurately reproduce the RP of 1,4-benzoquinone³⁶

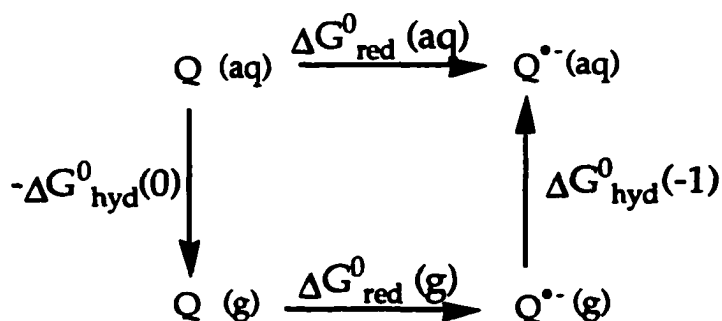


Figure 4-2. Thermodynamic Cycle 1 Used to Calculate the Aqueous One-Electron Reduction Potential of Quinones (Q) as a Sum of More Easily Accessible Free Energy Changes.

and a variety of halogenated quinone derivatives.² We use such a cycle in this study to calculate the reduction potentials of a selected set of quinones that model plastoquinone and ubiquinone, two of the common quinones used in biological electron transfer processes. For plastoquinone, we used 2,3,5,6-tetramethyl-1,4-benzoquinone, 2,3,5-trimethylquinone, and plastoquinone-1 as model compounds, while for ubiquinone, we used ubiquinone-1. These model compounds have been shown to provide excellent matches for the structural and spectroscopic properties of the parent compound,^{3,38,39} and duroquinone in particular has found widespread use as a redox indicator.⁴⁰

As can be seen in Figure 4-2, part of the cycle, $\Delta G^{\circ}_{\text{red}}(\text{g})$, requires the accurate calculation of the gas-phase one-electron reduction potential, or gas-phase EAs. Many methods have been used in attempts to calculate EAs to within chemical accuracy (a few kcal/mol), and any successful technique must account for extensive electron correlation involved in covalent bond changes, as well as the diffuse electron density found in anions. To meet these stringent requirements, such methods usually require prohibitive amounts of computational resources when applied to reasonably sized molecules. The recent appearance of more economical density functional (DF) and hybrid Hartree-Fock/density functional (HF/DF) methods led to the investigation of their potential in determining electron affinities. After testing a variety of functionals and basis sets,^{1,39} it was determined that a combination of the Becke3LYP (B3LYP) method and 6-311G(d,p) or 6-311G(3d,p) basis sets provides the most reliable results. Because the results for the two basis sets were virtually identical, we use the smaller of the two and present here the results of electron affinity determinations for thirteen different *para*-quinones and compare these results to experimental findings.^{33,41-43}

Of course, the EA properly approximates only the reduction enthalpy and neglects entropic contributions to the gas-phase reduction free energy. Published experiments nonetheless indicate that the gas-phase reduction enthalpy represents all but approximately 0.02 eV of the gas-phase reduction free energy for *p*-benzoquinones.^{33,41} The accuracy we obtained in these calculations (± 0.05 eV) makes this a reliable method for determining EAs, and it therefore provides us with excellent gas-phase data to use in the thermodynamic cycles in Figures 4-2 and 4-3 to determine RPs.

The free energy perturbation (FEP) method with molecular dynamics was chosen to model solvation energy changes because it yields accurate hydration free energy differences between various organic and biological molecules and between halide ions and noble gas atoms.⁴⁴⁻⁵² The FEP method was developed to minimize numerical errors by expressing the Hamiltonian, or energy operator, as a weighted sum of Hamiltonians for reactants and products. A more complete description of FEP is given in Section 1.3.3.

Simulations were thus performed for a constant pressure, temperature, and number of atoms. A temperature of 300 ± 20 K and a pressure of 1 atm were maintained by coupling the system to external temperature and pressure baths with a time constant of 0.1 ps.^{53,54} Bond distances were held constant by using the SHAKE coordinate re-setting algorithm^{55,56} to allow use of a 0.001 ps time step and all structures were equilibrated for at least 100 ps before beginning FEP calculations. In solution, a single molecule (ion) was studied in a rectangular box containing the molecule (ion) and 647 TIP3P water molecules,⁵⁷ with periodic boundary conditions. Interactions in solution were cut off beyond 10 Å and a Born charging correction⁵⁸⁻⁶⁰ of 0.711 eV was applied by using the first term from

equation 15 of reference 59, a multipole expansion of the energy for a distribution of charges within a sphere imbedded in a structureless, polarizable dielectric.^{61,62} Although the truncated multipole expansion is an approximation, higher-order terms in the expansion for quinone derivatives in water are very small, with the second term of the expansion (based on the dipole moment, here taken as 1 D to approximate the B3LYP/6-31G(d) 1.3 D dipole calculated for TMQ⁻) giving a value of about 4×10^{-4} eV, which is well beyond the accuracy of the methods employed here. Moreover, terms in the expansion of higher order than the dipolar term fall off as $1/r^5$ or faster, and therefore all terms from the second term on should be insignificant for the cutoff distance employed, and are therefore omitted in our calculations. The 10 Å radius of the sphere was used as the cutoff distance and the dielectric constant was assumed the same as the experimental dielectric constant of water, 78.

The Born charging correction reportedly recaptures approximately 95-98% of the hydration free energy lost by cutting off interactions between a single ion and distant water molecules.⁶³⁻⁶⁵ We have nonetheless investigated ways to attenuate the effects of cutoffs on calculated energy differences by considering thermodynamic cycles involving species of the same charge, such as that shown in Figure 4-3. The

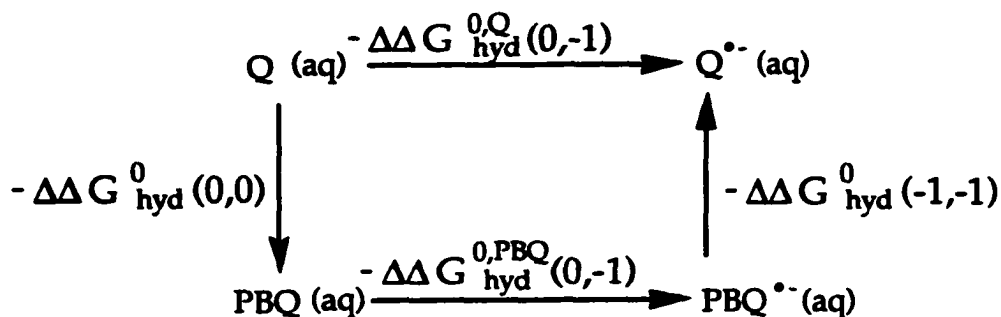


Figure 4-3. Thermodynamic Cycle 2 Used to Calculate the Reduction Potential of Various Quinones (Q) based on the Previously Determined Reduction Potential of 1,4-benzoquinone (PBQ).

simulations involved in calculating energies for the cycle shown in 3 were done similarly to those described above. FEP/MD simulations were accomplished by transforming one quinone into another and one semiquinone anion into the other, and the hydration free energy differences were combined according to the following equation to yield the reported hydration free energy difference between a substituted *p*-benzoquinone and its semiquinone anion.

$$\Delta\Delta G_{hyd}^{0,Q}(0,-1) = \Delta\Delta G_{hyd}^{0,PBQ}(0,-1) + (\Delta\Delta G_{hyd}^0(0,0) + \Delta\Delta G_{hyd}^0(-1,-1))$$

The calculated hydration free energy differences were then substituted into thermodynamic cycle 1 as

$$\Delta\Delta G_{hyd}^{0,Q}(0,-1) = \Delta G_{hyd}^0(-1) + \Delta G_{hyd}^0(0)$$

to estimate the one-electron reduction potential for the substituted quinones. Thus, by using the accurately calculated RP of PBQ, we avoid perturbing other quinones from neutral to charged states, and need not include the Born charging correction (since it is included already in the RP of PBQ). Gas phase perturbations were carried out in a fashion similar to that of the solution phase, except no periodic boundary conditions were applied, and there is no need for the Born charging correction since there is no solvent and all non-bonded interactions are calculated.

4.2 Results and Discussion

4.2.1 Electron affinities

Table 4-1 compares experimental electron affinities for a number of methylated and substituted 1,4-benzoquinones with electron affinities calculated by using the B3LYP/6-311G(d,p) method. Each individual calculated EA is within experimental error of its measured value. The correct ordering of the EAs, where experimental numbers are available, is reproduced by the calculated values with the exception of UQ0, which is discussed later. Calculations even reproduce the small difference in electron affinities between 2,5- and 2,6-dimethyl-*p*-benzoquinone. Where experimental values are unavailable, our calculated EAs represent predictions, the two most important being the prediction of a 1.68 eV EA for MQ1 and 1.75 eV EA for UQ0.

Table 4-1 shows that the trend of decreasing electron affinity with increased methyl substitution on the 1,4-benzoquinones is also displayed in the fused ring quinones. Adding a fused ring to PBQ (with a calculated electron affinity of 1.85eV) to form NQ reduces the electron affinity to 1.75eV, and adding

Table 4-1. Calculated (B3LYP/6-311G(d,p)) and Experimental Electron Affinities of Various Quinones.

Molecule	Abbr.	Experimental EA ^{33,41-43}	Calculated EA
9,10-anthraquinone	AQ	1.59	1.56
2,3,5,6-tetramethyl-1,4-quinone	DQ	1.62	1.63
2,3,5-trimethyl-1,4-quinone	TMQ	1.63	1.66
2,3-dimethyl-1,4-naphthoquinone	23NQ	-	1.63
Menaquinone-1	MQ1	-	1.68
2-methyl-1,4-naphthoquinone	2NQ	1.67-1.74	1.69
Menaquinone-1 (terminal hydrogens)	MQ	-	1.72
1,4-naphthoquinone	NQ	1.73-1.81	1.75
Ubiquinone-0	UQ0	1.86	1.75
2,3-dimethyl-1,4-quinone	23Q	-	1.74
2,5-dimethyl-1,4-quinone	25Q	1.76	1.69
2,6-dimethyl-1,4-quinone	26Q	1.77	1.70
2-methyl-1,4-quinone	2Q	1.79	1.77

another fused ring to form AQ decreases the electron affinity by a further 0.2eV to 1.56eV. This reflects the trend found in the experimental numbers. What is perhaps unusual is that the calculated electron affinity of MQ-1 (1.68eV) is only 0.01eV lower than that of 2NQ (1.69eV) and 0.05eV higher than the predicted value of 1.63eV for 23NQ. While the calculated electron affinities of 2NQ, MQ, and 23NQ are essentially equal within the error of the calculation (~ 0.05 eV), we note that our study finds that a change of the isoprenoid chain-terminal methyl groups on MQ1 into hydrogens (the model compound MQ, tried for computational efficiency) actually raised the electron affinity to 1.73eV. These differences seem to imply that although the isoprenoid chain has the same structural effect on the ring as a methyl group (as discussed previously in Section 4.1), its electronic effect is different, perhaps owing to some long-range "communication" between the double bond in the chain with the ring system. Considering that most naturally occurring forms of menaquinone have four or nine isoprenoid chain units, and that the conformations of substituents may play a role in determining the electron affinity,⁶⁶ it may be interesting to explore the effects of lengthening the chain or changing its orientation on the electron affinity, but earlier work with plastoquinone-1³ and ubiquinone-1^{38,39} seems to indicate that it has only a minimal impact for reasonable conformations. However, the 0.11 eV overestimation of the EA for UQ0 is unexplained. (See Section 4.2.2 for a discussion of how this EA difference may be involved in the final RP calculations.)

Because we were interested in determining the most computationally efficient method for accurately determining EAs, we tried a second method of predicting the electron affinities on some of the largest molecules (NQ, 2NQ,

23NQ, and AQ). The first method involves a full optimization of both the neutral species and its radical anion at the B3LYP/6-311G(d,p) level (the results in Table 4-1). The second method involves only the energy differences in single-point calculations at the B3LYP/6-311G(d,p) level on optimized structures from B3LYP/6-31G(d) calculations. In two cases, NQ and 23NQ, the electron affinity was unchanged from one method to another. In the other two cases, AQ and 2NQ, the electron affinity decreased by 0.01 eV and 0.03 eV, respectively, and in both cases moved away from experimental values. So this simpler method may be useful in determining the EA of larger species, but more work needs to be done since in two of the four tests here, the calculated EA moved further from experiment.

It should be noted that the geometries obtained with the B3LYP method during EA calculations are very close to all experimental data. For a detailed discussion of the B3LYP method's ability to reproduce structural and spectroscopic properties of quinones, the reader is referred to Chapters 2 and 3 and the references therein.

4.2.2 Reduction potentials

Table 4-2 reports the calculated aqueous one-electron reduction potentials of four quinones representative of some of the biologically important species. As discussed in Section 4.1, we used a second thermodynamic cycle (Figure 4-3) to determine the RP of DQ and TMQ, the two whose RP from cycle 1 was further from experiment (0.19 and 0.17 eV, respectively), and these results are also reported in Table 4-2. Clearly, the use of thermodynamic cycle 2 did not improve the results, and actually gave RPs further from experimental values. Cycle 1,

Table 4-2. Calculated Reduction Potentials (RPs) for Selected Quinones. All values are reported in eV. Cycle 1 and cycle 2 are different thermodynamic cycles (see Figures 4-2 and 4-3) used to calculate separate RPs. The RP for a particular cycle is determined by adding the respective hydration free energy difference, $\Delta\Delta G^{\circ}_{\text{hyd}}$, and the calculated electron affinity (EA).

Redox Pair	$\Delta\Delta G^{\circ}_{\text{hyd}}{}^{\text{a}}$ cycle 1 / cycle 2	Calculated EA	Calculated RP cycle 1 / cycle 2	Experimental ^b RP ^{67,79}
DQ/DQ ^{•-}	2.36 / 2.32	1.63	3.99 / 3.95	4.18-4.21
TMQ/TMQ ^{•-}	2.45 / 2.36	1.66	4.11 / 4.02	4.28
PQ1/PQ1 ^{•-}	2.47 / -	1.75 ³	4.22 / -	4.28
UQ1/UQ1 ^{•-}	2.35 / -	1.81 ³⁹	4.16 / -	4.20

^a These values incorporate a Born charging correction of 0.7090 eV (see the text for a full discussion).

^b Experimental reduction potentials obtained by adding 4.44 eV to the reported value to correct for the standard hydrogen electrode.

however, gave very good results for PQ1 and UQ1, with RPs differing from experiment by only 0.06 and 0.04 eV, respectively.

We confirmed the convergence of the simulations by running independent calculations for increasing total time lengths, ranging from about 25ps to 150ps. We also checked our simulations by running independent calculations in the reverse direction, and averaging the forward and reverse quantities to determine energy changes. The difference in the forward and reverse energies (which are all less than 0.05 eV at longer simulation times) may be taken as a lower limit on the error in the simulation. A detailed breakdown of the free energy differences obtained in each calculation is reported in the Supplemental Information section in Tables S4-1 through S4-4.

The calculated aqueous one-electron reduction potential for PQ1 is only 0.06 eV less than the experimental value of 4.28 eV,⁶⁷ but it is 0.11 eV higher than the reduction potential calculated for TMQ. Since the experimental reduction potentials for TMQ and PQ1 are identical, our calculations show a discrepancy between the two calculated RPs which is 0.11 eV, slightly larger than the error range from other calculated reduction potentials (~0.1 eV), but less than the error found in TMQ and DQ (~0.18 eV). The source of the difference in the calculated RPs for TMQ and PQ1 is currently unknown, but because the calculated hydration free energy differences between TMQ and TMQ⁻ (2.45 eV) and PQ1 and PQ1⁻ (2.47 eV) are almost identical, the problem would seem to be with the electron affinities. As mentioned earlier, previous work indicates that the conformation of the isoprenoid tail may affect the electron affinity,⁶⁶ and therefore the minimum energy geometry found for PQ1³ and used here for EA and RP calculations may need to be reevaluated. And, since we have seen above that the EA of at least one

molecule, UQ0, is overestimated by exactly the same amount that the RPs of TMQ and PQ1 differ, it must be considered that a cancellation of errors can result in exceptionally accurate calculated RPs, such as those for PQ1 and UQ1. Normally, the thermodynamic cycle method can be expected to provide very accurate reduction potentials within about 5% (or within about 0.20 eV) of experimental values.

Table 4-2 demonstrates that hydration free energy differences make a larger contribution than gas-phase electron affinities to the Gibbs free energy of reduction for the quinones studied. Because hydration free energy differences should depend on the extent of solvent reorientation upon going from the neutral quinone to the reduced semiquinone anion, we have investigated changes in hydration structures by analyzing radial distribution functions^{68,69} for DQ as a simple, symmetric example. Various factors influence solvent structure, but we note that our simulations include no special parameters to describe hydrogen bonding, so any hydrogen bonding interactions must arise from a combination of electrostatic and Lennard-Jones interactions between solute-solvent and solvent-solvent atom pairs.

Radial distribution functions were calculated from structural data gathered every 10 fs over a 50 ps equilibrated MD run. The data showed a very clear solvation shell around the oxygens in the quinone radical anion with water hydrogens about 1.8 Å away, and water oxygens about 2.8 Å away. Integration of the radial distribution functions showed that an average of about 3 water molecules were present in this first solvation shell, and due to the average linear geometry of the DQ⁻, O-water, H-water geometry, it seems clear that they may be considered to be hydrogen bound, and this conclusion is further supported by crystallographic

descriptions of hydrogen bonding.⁷⁰⁻⁷² As a further check, we performed energy analysis and found that on average, all waters in this first solvation shell interacted with the quinone with an energy of at least 2.25 kcal/mol, a value which has been used as an energetic definition of a hydrogen bond by Jorgensen et al.^{57,73-75} The results of solvation analysis on the neutral DQ molecule showed a much less pronounced first solvation shell, at slightly further distances from the quinone oxygens, made up of less than 2 water molecules on average and the hydrogens and oxygens of water closest to the quinone oxygens are located no closer than the sum of the van der Waals radii. Furthermore, the water hydrogens adopt poorly defined geometries and indicate little orientational ordering. In summary, stronger hydrogen bonding to the oxygens of quinone radicals appears to make the largest contribution to hydration free energy differences between the quinones.

4.3 Conclusions

One-electron reductions are fundamental reactions in virtually every sub-discipline of chemistry, yet only recently have one-electron reduction potentials^{36,37} and potential differences between two different molecules^{46,76-78} been calculated from atomic properties of solutes and solvents. This contribution tests a method recently proposed for computing aqueous reduction potentials⁵⁰ by applying the method to the biologically important electron acceptors plastoquinone and ubiquinone, as well as model compounds 2,3,5-trimethyl-1,4-benzoquinone and 2,3,5,6-tetramethyl-1,4-benzoquinone. The method uses thermodynamic cycles to express the reduction free energy as a sum of the gas-phase reduction free energy plus the hydration free energy difference between the neutral molecule and its radical anion. Part of the cycle requires the accurate calculation of gas

phase electron affinities, and we have demonstrated that the B3LYP/6-311G(d,p) method can yield electron affinities within experimental error for the quinones tested and within an average absolute magnitude of 0.05 eV of experimentally measured electron affinities. We also offer predicted electron affinities of several quinones for which no experimental EA has been measured.

The most accurate computed one-electron reduction potentials are within 40 to 270 meV of experiment. The least accuracy was obtained for TMQ ($RP_{\text{calc}} = 4.11$ eV and $RP_{\text{expt}} = 4.28$ eV) and DQ ($RP_{\text{calc}} = 3.99$ eV and $RP_{\text{expt}} = 4.18$ to 4.21 eV). While for the compounds most relevant to biological electron transfer, plastoquinone and ubiquinone, very good agreement with experiment was obtained (RP_{calc} for PQ1 = 4.22 eV and $RP_{\text{expt}} = 4.28$ eV, while RP_{calc} for UQ1 = 4.16 eV and $RP_{\text{expt}} = 4.20$ eV). Because the errors in calculated electron affinities are relatively small, the FEP/MD simulations represent the largest source of error. Nonetheless, relative magnitudes of calculated electron affinities, hydration free energy differences, and reduction free energies were well reproduced. An analysis of the average solvation structure showed that energetic features and quinone oxygen-water hydrogen distances and linear O---H-O geometries are consistent with hydrogen bonds to the radical anions, while there is much less structural ordering around the neutral molecules.

Although the relatively good agreement between experimental and calculated EAs and the RPs for a variety of quinones confirms the plausibility of combining thermodynamic cycles with hybrid HF/DF quantum chemical and FEP/MD methods to estimate one-electron reduction potentials, the next phase of the study would be to demonstrate the method's effectiveness in a variety of solvents and in proteins.

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Table S4-1. Detailed Results of the Reduction Potential Calculations of 2,3,5,6-tetramethyl-1,4-benzoquinone (DQ). All values are reported in eV. See Chapter 4 for a detailed discussion of the results.

Perturbation	Phase	2.5ps/window	5ps/window	15ps/window
DQ->DQ ^{•-}	soln	-0.6007	-0.6932	-0.6860
	gas	0.9538	0.9597	0.9507
DQ ^{•-} ->DQ	soln	0.7083	0.7008	0.6708
	gas	-0.9644	-0.9481	-0.9673
DQ->PBQ	soln	-0.9112	-0.9453	-0.9417
	gas	-0.9983	-0.9847	-0.9845
PBQ->DQ	soln	0.9776	0.9463	0.9827
	gas	0.9779	0.9763	0.9840
DQ ^{•-} ->PBQ ^{•-}	soln	-1.847	-1.809	-1.817
	gas	-1.481	-1.479	-1.515
PBQ ^{•-} ->DQ ^{•-}	soln	1.803	1.810	1.845
	gas	1.490	1.485	1.502

Corresponding One-Electron Reduction Potentials of TMQ				
Cycle 1		3.96	4.00	3.99
Cycle 2		3.98	3.95	3.94

Table S4-2. Detailed Results of the Reduction Potential Calculations of 2,3,5-trimethyl-1,4-benzoquinone (TMQ). All values are reported in eV. See Chapter 4 for a detailed discussion of the results.

Perturbation	Phase	2.5ps/window	5ps/window	15ps/window
TMQ->TMQ ⁻	soln	-0.8325	-0.8191	-0.8565
	gas	0.8811	0.8817	0.8817
TMQ ⁻ ->TMQ	soln	0.8894	0.8734	0.8584
	gas	-0.8816	-0.8817	-0.8828
TMQ->PBQ	soln	-0.4610	-0.4694	-0.4676
	gas	-0.4703	-0.4757	-0.4767
PBQ->TMQ	soln	0.4377	0.4239	0.4430
	gas	0.4723	0.4706	0.4684
TMQ ⁻ ->PBQ ⁻	soln	-1.102	-1.097	-1.116
	gas	-0.8954	-0.8886	-0.8960
PBQ ⁻ ->TMQ ⁻	soln	1.091	1.061	1.107
	gas	0.8915	0.8940	0.8862
Corresponding One-Electron Reduction Potentials of TMQ				
Cycle 1		4.11	4.10	4.11
Cycle 2		4.03	4.04	4.02

Table S4-3. Detailed Results of the Reduction Potential Calculations of Plastoquinone-1 (PQ). All values are reported in eV. See Chapter 4 for a detailed discussion of the results.

Perturbation	Phase	2.5ps/window	5ps/window	15ps/window
PQ->PQ ⁻	soln	-0.8939	-0.8467	-0.8839
	gas	0.8995	0.8993	0.8783
PQ ⁻ ->PQ	soln	0.9282	0.9005	0.8558
	gas	-0.9032	-0.9068	0.8996

Corresponding One-Electron Reduction Potentials of TMQ				
Cycle 1		4.27	4.24	4.22

Table S4-4. Detailed Results of the Reduction Potential Calculations of Ubiquinone-1 (UQ). All values are reported in eV. See Chapter 4 for a detailed discussion of the results.

Perturbation	Phase	2.5ps/window	5ps/window	15ps/window
UQ->UQ ^{•-}	soln	-0.4619	-0.4410	-0.4702
	gas	1.223	1.228	1.205
UQ ^{•-} ->UQ	soln	0.4151	0.4751	0.3906
	gas	-1.217	-1.220	-1.210
Corresponding One-Electron Reduction Potentials of TMQ				
Cycle 1		4.18	4.20	4.16

CHAPTER 5

The Binding Sites of Q_B and $Q_B^{\cdot-}$ in the Photosynthetic Reaction Center of *Rhodobacter sphaeroides* determined by Molecular Dynamics

5.1 Introduction

A fundamental understanding of the step-by-step process of photosynthesis is still elusive in part because the atomic-level three-dimensional structure of photosystems I and II are currently unknown.¹ Bacterial photosynthetic reaction centers, such as that from the purple bacterium *Rhodobacter sphaeroides*, are useful as models of the plant photosystem^{2,3} in part because numerous atomic-scale X-ray structures of these systems have been described in detail.^{4,5} Even in these simpler systems, unanswered questions remain. After electron transfer from the primary donor (the special pair) to the primary quinone (Q_A), the electron is then transferred to a secondary quinone, Q_B ,⁶⁻¹⁴ which in the case of *Rb. sphaeroides* is ubiquinone-10.¹⁵⁻¹⁷ This first electron transfer between quinones has been the subject of many experimental studies. Some studies have shown that the rate limiting step in $Q_A^{\cdot-} \rightarrow Q_B^{\cdot-}$ electron transfer has an activation energy of about 15 kcal/mol.^{18,19} The exact intermediate reactions that dictate the activation energy are not known. Recent experimental studies,²⁰ including a report of the X-ray structure of the reaction center in the charge-separated state,²¹ have prompted speculation that there are two possible binding sites for the secondary quinone. Stowell et al.²¹ have speculated that movement of Q_B from one site to the other could be responsible for the observed activation energy of electron transfer. This

idea is reinforced by five previous X-ray structures of the RC at various resolutions, four of which find Q_B in one site,^{8,22-24} (PDB reference codes 4RCR, 2RCR, 1PSS, and 1YST) while the fifth²⁵ (PDB reference code 1PCR) binds Q_B about 5 Å away.⁴ One of these sites may be more conducive to electron transfer than the other, and the movement of Q_B (or Q_B^{•-}) between the sites may be necessary before Q_A^{•-} → Q_B^{•-} electron transfer is complete. Much of what is currently known about the binding of Q_B^{•-} in *Rb. sphaeroides* has been inferred from vibrational spectroscopy,²⁶⁻³⁹ and it has been well established that the first electron transfer to Q_B is not accompanied by proton transfer to the quinone.⁴⁰⁻⁴³ However, it is also clear that electron transfer to Q_B is accompanied by proton uptake by the reaction center protein and trapping of the proton near Q_B,^{18,20,34,44-54} and the reaction is known to be pH dependent.^{10,11,55}

The Q_B binding site contains two nearby amino acids (GLU L212, which is conserved in all plant and bacterial systems, and ASP L213, where the “L” designates that the residue is in the L protein subunit) whose protonation states may vary, and may have profound effects on the local electrostatic environment of Q_B and the thermodynamics of electron transfer. Several site-directed mutational studies have demonstrated the importance of these two residues,^{3,56-64} and in another recent study, Paddock et al. suggested that in the neutral, ground state of the RC, GLU L212 is predominantly protonated, while ASP L213 is predominantly ionized.⁶⁵ It is known that electron transfer rate between the quinones is not dependent on the reduction potential of the quinone at Q_B, but is significantly affected by pH.^{10,11,55} This implies that the actual electron transfer is not the rate limiting step, so the notion of two binding sites, one of which is

“activated”, or conducive to electron transfer, is consistent with this data, but the pH dependence is not obviously accounted for in this theory.

Computational chemistry offers ways to explore the events associated with photosynthesis at the atomic level in controlled, known conditions nearly impossible to achieve experimentally. Methods ranging from molecular dynamics to semi-empirical and mixed quantum mechanics/molecular mechanics have been applied to various photosynthetic reaction centers.⁶⁶⁻⁷⁸ None of these studies have attempted to address the specific binding site of Q_B in *Rb. sphaeroides*. We present here a series of molecular dynamics studies on the photosynthetic reaction center of *Rb. sphaeroides* which supports the existence of two binding sites for Q_B. We describe the average binding site of Q_B in both the neutral and radical anionic forms in each of four possible nearby amino acid protonation states: L212 and L213 both ionized, GLU/ASP; L212 protonated and L213 ionized, GLH/ASP; L212 ionized and L213 protonated, GLU/ASH; and L212 and L213 both protonated, GLH/ASH. We compare these data with the available crystal structures. Our results show that the protonation state of these two amino acids has a significant effect on the binding site of Q_B, and that the two different binding sites identified experimentally are likely due to a change in the L212/L213 protonation state. The GLH/ASP state corresponds well with the inactivated, neutral site reported by Stowell et al. and also identified by Ermler et al., while the GLH/ASH state corresponds to the activated, neutral binding site and the binding site of Q_B^{•-} identified by Stowell et al., and reported as the Q_B binding site by others. Based on these results we suggest that either (1) the structures reported by Stowell et al. actually show the different binding sites of the neutral quinone when the nearby amino acids are in different protonation states, or (2) that if the work of

Stowell et al. actually does show the binding site of the quinone radical anion, the movement of the quinone between these two binding sites is, by itself, not sufficient to explain the activation energy, but that it is instead likely the result of the changing protonation state of ASP L213.

5.2 Simulation Details

5.2.1 General Information

The RC model was constructed based on the X-ray structure of Ermler et al.,²⁵ and contains 823 amino acid residues, 4 bacteriochlorophylls, 2 bacteriopheophytins, 2 ubiquinones, 1 non-heme iron, and 160 crystallographic waters. This starting geometry was chosen for its relatively high resolution (2.65 Å) and large number of identified water positions. The quinone isoprenoid tails were located only partially, and the remaining isoprene units were added as described below in Section 5.2.3 to create UQ10. The TIP3P model⁷⁹ was used for all waters. The AMBER^{80,81} suite of programs were used for all calculations. A non-bonded interaction cutoff of 15 Å was applied to the system, and a moving belly was used that allowed only the residues in the system with any atom within 15 Å of any atom of either quinone to move. (Other non-bonded and belly cutoffs were tested, ranging from 10 Å to 20 Å, but the results appeared insensitive to the larger cutoffs. The smaller, 10 Å cutoff was found to be too short since in some cases, Q_B was between 10 Å and 15 Å away from the non-heme iron, and this interaction may be important.) The moving belly contained 4514 heavy atoms and polar hydrogens out of a total of 8785. A spherical harmonic restraint, or “cap”, with a 50 Å radius and a 1.5 kcal/mol force constant was centered at the center of

mass of the two quinones and applied to all mobile water molecules to prevent their “evaporation” from the system. No periodic boundary conditions were applied and a constant temperature of $300\pm 20\text{K}$ was maintained by the Berendsen coupling algorithm.⁸² All bond distances were held constant using the SHAKE algorithm^{83,84} which allowed the use of a 0.001 ps time step. All simulations were carried out with either Q_A or Q_B in the radical anion state.

Each simulation was started from the X-ray coordinates after a 200 step steepest-descent minimization. All simulations were equilibrated for at least 200 ps (total energy and temperature drift both indicated that the systems were at equilibrium, see Supplemental Tables S5-1 and S5-2), and then structural “snapshots” were taken once every 50 fs during a further 75 ps equilibration for averaging. The average structures over these 1500 snapshots was then analyzed as described below. Tables 5-1 through 5-3 give a comparison of the RMSD between the available experimental structures and between our calculated average structures and the most recent X-ray structures. The average RMSD in the position of the backbone atoms between experimental structures is about 1.5 Å, whereas our calculated average structures differ from recent experimental structures by 1.1 Å or less. This indicates that our average structures are reasonable representations of the reaction center. The quinones themselves generally moved less than about 0.5 Å over the entire 75 ps of data analyzed.

5.2.2 The Protein Residues

All 823 protein residues found in the X-ray structure of Ermler et al. were used in this simulation. The charges and force fields of the protein are taken from a standard AMBER united-atom force field⁸⁵ except as otherwise noted below.

Table 5-1. RMSD (Å) in the Position of the Backbone Atoms in Six Previous Experimental X-ray Structures of the *Rb. sphaeroides* Reaction Center.

	1AIJ ²¹	1PCR ²⁵	1PSS ²³	1YST ²⁴	2RCR ²²	4RCR ¹
1AIJ	0.00	0.52	1.40	1.58	1.90	1.50
1PCR		0.00	1.41	1.56	1.85	1.51
1PSS			0.00	1.82	1.91	0.99
1YST				0.00	1.50	1.86
2RCR					0.00	1.90
4RCR						0.00

Table 5-2. RMSD (Å) in the Position of the Backbone Atoms Between Average Calculated Structures in the Q_A⁻/Q_B State and Two Recent X-ray Structures.

	1AIJ ²¹	1PCR ²⁵
GLU/ASP	0.99	0.90
GLH/ASP	1.14	1.07
GLU/ASH	0.98	0.90
GLH/ASH	0.96	0.88

Table 5-3. RMSD (Å) in the Position of the Backbone Atoms Between Average Calculated Structures in the Q_A⁻/Q_B State and the Stowell et al. Charge Separated State X-ray Structure.

	1AIG ²¹
GLU/ASP	1.09
GLH/ASP	1.10
GLU/ASH	1.02
GLH/ASH	1.03

This force field explicitly includes polar hydrogens, and a special term for hydrogen bonding. This H-bonding term applies only between amino acids, and *not* between amino acids and quinones. Unless otherwise noted, all amino acids are assumed to be in the protonated state appropriate for pH 7.0. In the case of GLU L212 and ASP L213, whose protonation state is varied in these simulations, charges for the protonated united-atom residues (not included in standard AMBER united atom force field) are derived by taking the residue models from an all-atom AMBER force field⁸⁶ and adding the charges of hydrogens into heavy atoms, except in the case of the carboxyl group, where the polar hydrogen is included specifically.

5.2.3 The Quinones

The equilibrium geometries of the quinone head-group in ubiquinone-10 were derived from B3LYP/6-31G(d) calculations on ubiquinone-1 and its radical anion by Boesch and Wheeler.^{87,88} Charges, force constants, and atom types were derived according to the procedure outlined in Chapters 1 and 4. This method of quinone force field derivation was shown to give very good results for the aqueous one-electron reduction potentials of a variety of quinones (including a calculated reduction potential of 4.16 eV for ubiquinone-1 vs. an experimental value of 4.20 eV discussed in Chapter 4), a property which was shown to be related to the solvation structure of the nearby waters induced by the quinone force field.⁸⁹ Based on this evidence, we therefore have confidence in the ability of our quinone force fields to correctly model the interactions of UQ10 (which was used in all simulations) with the surrounding protein in the RC. It should be noted that no special parameters are included to induce hydrogen bonding between the quinone

and polar hydrogens, so any such interaction noted in the simulation will be due to a combination of electrostatic and Lennard-Jones interactions.

We performed separate B3LYP/6-31G(d) calculations on isolated one-unit and two-unit isoprenoid chains to derive appropriate charges for these structures in UQ10. The charges of the first unit of the chain are left unchanged from the UQ1 model described in Chapter 4, so for the radical anion, all of the -1 charge is located on the quinone head group and first isoprene unit. Each additional unit in the chain is given a net zero charge, based on CHelpG⁹⁰ charges derived from the B3LYP/6-31G(d) calculations, so that the same force field may allow the modeling of ubiquinones with arbitrarily long isoprenoid chains.

5.2.4 The Chromophores

For the purposes of this simulation, we assumed that the special pair had already been reduced to its neutral form by cytochrome *c*, and therefore each BCL and BPH residue has an overall neutral charge. However, the BPH residues are within the 15 Å cutoff used in the simulation, and future simulations may require accurate partial atomic charges for all chromophores. Therefore, we invested a great deal of effort to generate reasonable charge distributions by fully optimizing the structures of models of BCL and BPH in various charge states at the B3LYP/3-21G level.⁹¹ The CHelpG charge-fitting algorithm⁹⁰ was used to determine partial charges, and a complete list of the charges used in RC simulations may be found elsewhere.⁹¹ For partial charges on the special pair, we performed a B3LYP/3-21G single-point calculation on two BCL molecules (each in the geometry of the optimized, isolated molecule) placed at a distance and orientation similar to that found experimentally for the special pair in Rb sphaeroides RCs. In all cases, the

charges for the isoprenoid tails are those that were derived for the quinones as described in Section 5.2.3. For simplicity, the Lennard-Jones parameters used in the chromophores were chosen from similar atom types in the standard AMBER united-atom force field.

5.2.5 The Non-Heme Iron

The non-heme iron is almost directly between the binding sites of Q_A and Q_B, and it is known to be involved in the electron transfer between quinones.^{92,93} It is complexed by four histidine residues (L190, L230, H219, and H266) and one glutamate (M234) in a pseudo-octahedral geometry. The -1 charge of the glutamate and formal +2 charge of the iron give the overall complex a +1 charge. Because the iron complex is about 10 Å away from each quinone, well within the 15 Å cutoff used for non-bonded interactions, it is important that we model its charge as accurately as possible. The formal charge of +2 is unlikely to be realistic, so we performed a single-point B3LYP/3-21G calculation on the iron and modified ligands. We modified the ligands by removing any atoms further than four bonds away from the iron, effectively modeling the histidines with 5-methylimidazole, and the glutamate with deprotonated propionic acid. The complex was oriented according to X-ray coordinates,²⁵ and hydrogens were added where appropriate. Because the CHelpG⁹⁰ charge-fitting algorithm (which we used to derive other charges) may give unreliable results for atoms deeper inside the complex, and because the central iron charge is quite important in this case, we elected to use Mulliken⁹⁴ charges in the simulation. From the results of the B3LYP/3-21G calculation, we estimate the iron charge at 0.8500, while the charges of the histidines are 0.1375 each, and that of the glutamate residue is

-0.4000. The charges of the omitted parts of each residue were unmodified from the original AMBER force field,⁸⁵ and so the overall charge on the non-heme iron complex remains +1. For the simulation, a bond was defined between each ligand and the iron (with bond distances as reported in the Ermler et al. X-ray structure) in order to prevent iron drift, which was noted to occur otherwise.

5.3 Results and Discussion

5.3.1 Neutral Q_B Binding

In the neutral state, various groups have reported Q_B binding sites that vary over a range of 5-7 Å, with some of the positions near H-bonding distance to HIS L190. Stowell et al.²¹ has recently published high resolution structures of the RC both before and after charge separation, and we will now discuss how these results are consistent with previous X-ray structures and our calculated binding sites.

In Table 5-4, we report distances from various H-bond donors to the proximal (O1) and distal (O4)--relative to the isoprenyl side chain (see Figure 4-1)--carbonyl oxygens of Q_B in RC with GLU L212 and ASP L213 in various protonation states. Figure 5-1 gives a comparison of the positions of the Q_B quinone head groups from experiments^{8,22-24} and from our calculated average structures (the models are aligned by a best-fit for the L190, L212, and L213 residues). It is first worth noting that the variance in the binding site of Q_B determined theoretically for different amino acid protonation states is very similar to the experimental situation. This may indicate that the different

Table 5-4. Calculated Distances (Å) of Neutral Ubiquinone (Q_B) Carbonyl Oxygens to Selected Potential Hydrogen Bond Donors for Different Protonation States of GLU L212 and ASP L213 Compared to X-ray Results.

Atoms	Protein Residue	X-ray ²¹	GLU/ASP	GLH/ASP	GLU/ASH	GLH/ASH
O1-N	L182	9.99	8.75	11.31	17.36	14.38
O1-N	L185	7.68	6.69	9.17	14.31	11.69
O1-N	L186	4.89	4.10	6.63	11.70	8.93
O1-N	L187	5.67	5.65	7.51	10.66	8.34
O1-N	L189	5.51	5.68	5.75	9.60	8.07
O1-N	L190	4.72	5.69	5.26	7.08	5.57
O1-ND1	L190	6.72	8.16	7.43	4.49	4.62
O1-OE1	L212	11.51	11.39	11.05	4.56	6.96
O4-ND1	L190	9.20	9.00	8.48	9.53	9.45
O4-OD1	L213	8.66	5.88	5.18	3.10	2.87
O4-N	L216	7.90	5.72	6.65	7.07	5.61
O4-N	L220	8.34	7.58	7.96	11.70	8.61
O4-N	L221	8.12	7.97	7.85	10.81	8.46
O4-N	L222	6.27	6.68	6.16	8.89	6.84
O4-OG	L223	5.53	3.66	3.70	7.01	5.64
O4-N	L223	4.43	4.19	3.79	6.67	4.95
O4-N	L224	3.14	4.37	3.63	4.21	3.23
O4-N	L229	7.11	7.65	7.26	6.84	6.75
Avg. Abs. Difference from X-ray		-	0.93	1.07	3.64	2.10

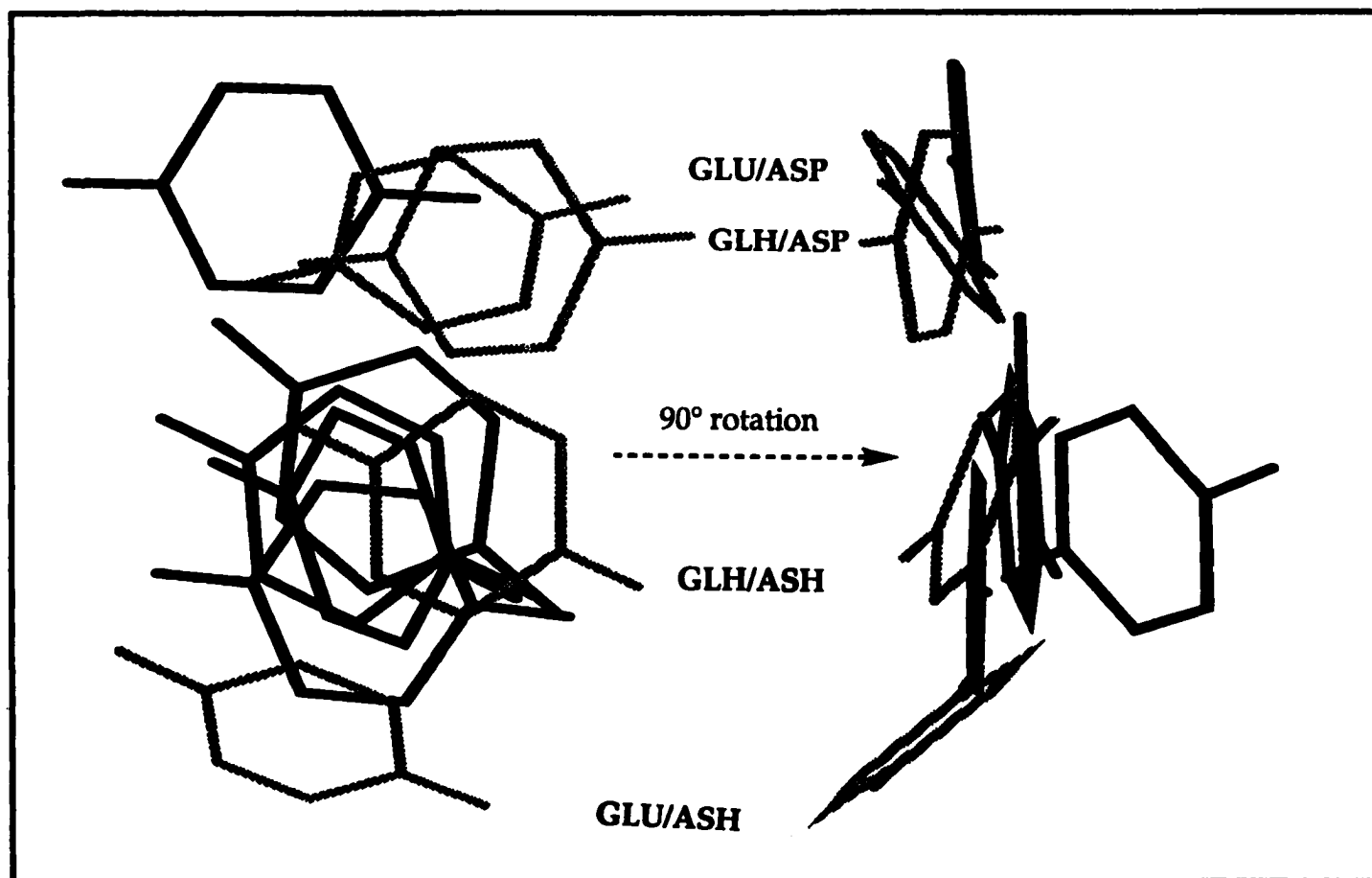


Figure 5-1. A Comparison of the Locations of the Secondary Quinone in the *Rb. sphaeroides* Reaction Center as Determined in Previous Experimental X-ray Structures^{8,22-25} (black) and in Our Calculations (grey) with GLU L212 and ASP L213 in Different Protonation States. Two views, rotated 90° apart, are shown. All substituents except the carbonyl groups have been removed.

locations of Q_B observed experimentally are the result of differing protonation states of L212 and L213. We can see from Figure 5-1 that four of the previous X-ray structures^{8,22-24} are grouped together in a location similar to that calculated to result from the GLH/ASH protonation state. In all four of these X-ray structures, the proximal carbonyl group of Q_B (the one closest to the isoprenyl chain) is farthest from HIS L190. Our calculated binding site for the GLH/ASH state does not reproduce this orientation, instead we find the proximal carbonyl to be nearest L190, roughly a 180° rotation about the isoprenyl chain. In the X-ray structure by Ermler et al.²⁵ (the starting point of each of our calculations), the quinone was flipped 180° and moved away from L190, and was in a location and orientation very similar to that predicted by our simulations for the GLU/ASP and GLH/ASP protonation states.

The recent structure determination reported by Stowell et al. shows a Q_B binding site very similar to that reported by Ermler et al. Table 5-4 gives a comparison of the distances from the carbonyl oxygens of Q_B to various nearby hydrogen bond donors from both Stowell et al.'s X-ray structure and our predicted binding sites for various protonation states. Figures 5-2 through 5-5 also give a graphical representation of the quinone position relative to HIS L190, GLU L212, and ASP L213. From these data, it is quite clear that the structures predicted by molecular dynamics for the GLU/ASP and GLH/ASP protonation states are quite similar to the results of Stowell et al. for the neutral, inactivated location. The binding site predicted for Q_B in the GLU/ASH state does not correspond to any previously determined experimental site (see Figure 5-1). These findings are consistent with previous work that found ASP L213 to have a much lower pK value, by about 5 pK units, than GLU L212, and therefore much more likely to be

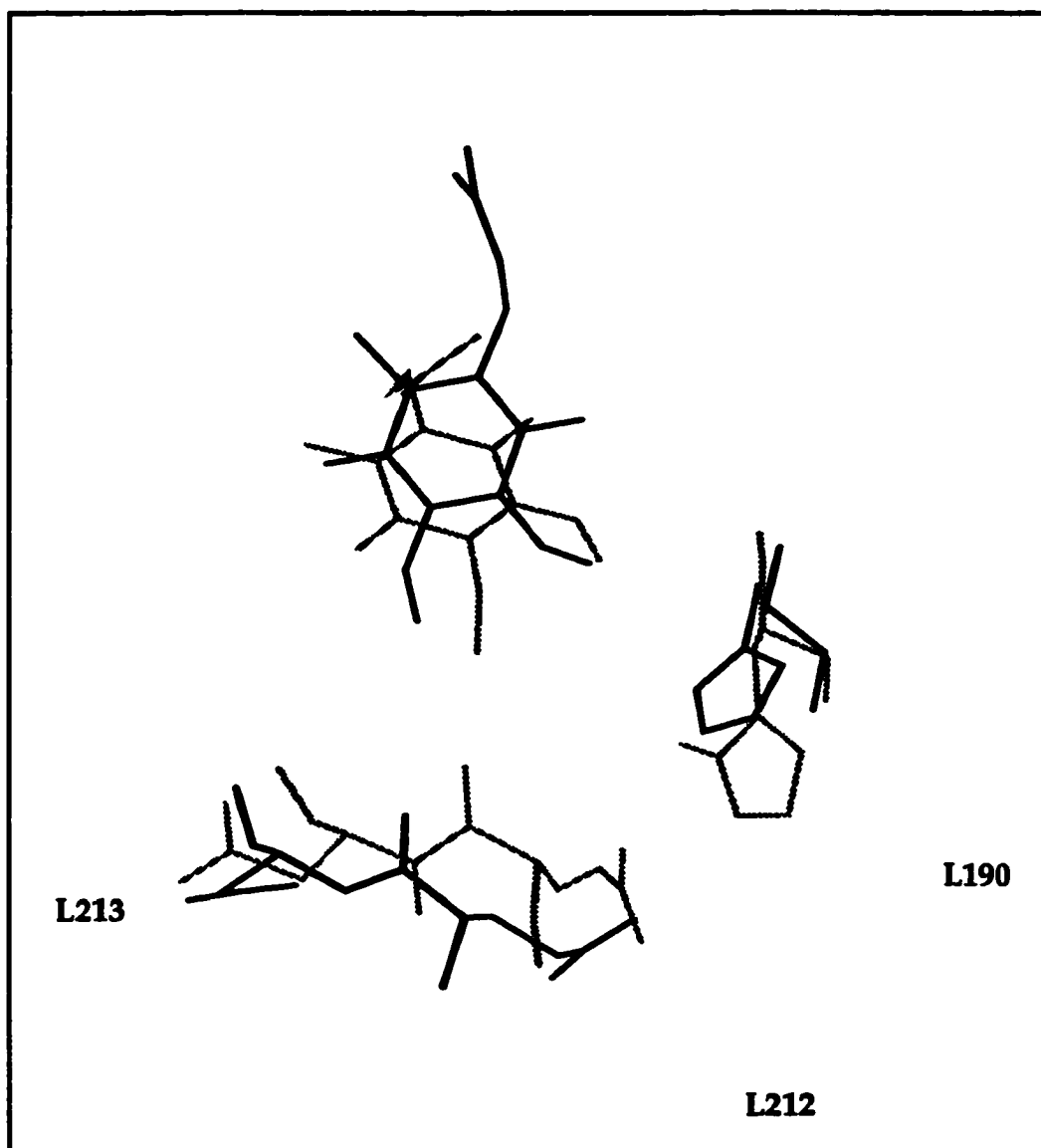


Figure 5-2. A Comparison of the Calculated Average Position of Neutral Q_B in the GLU/ASP Protonation State (grey) with an Experimentally²¹ Determined Position (black). The quinone is predicted to be quite near the experimentally observed location.

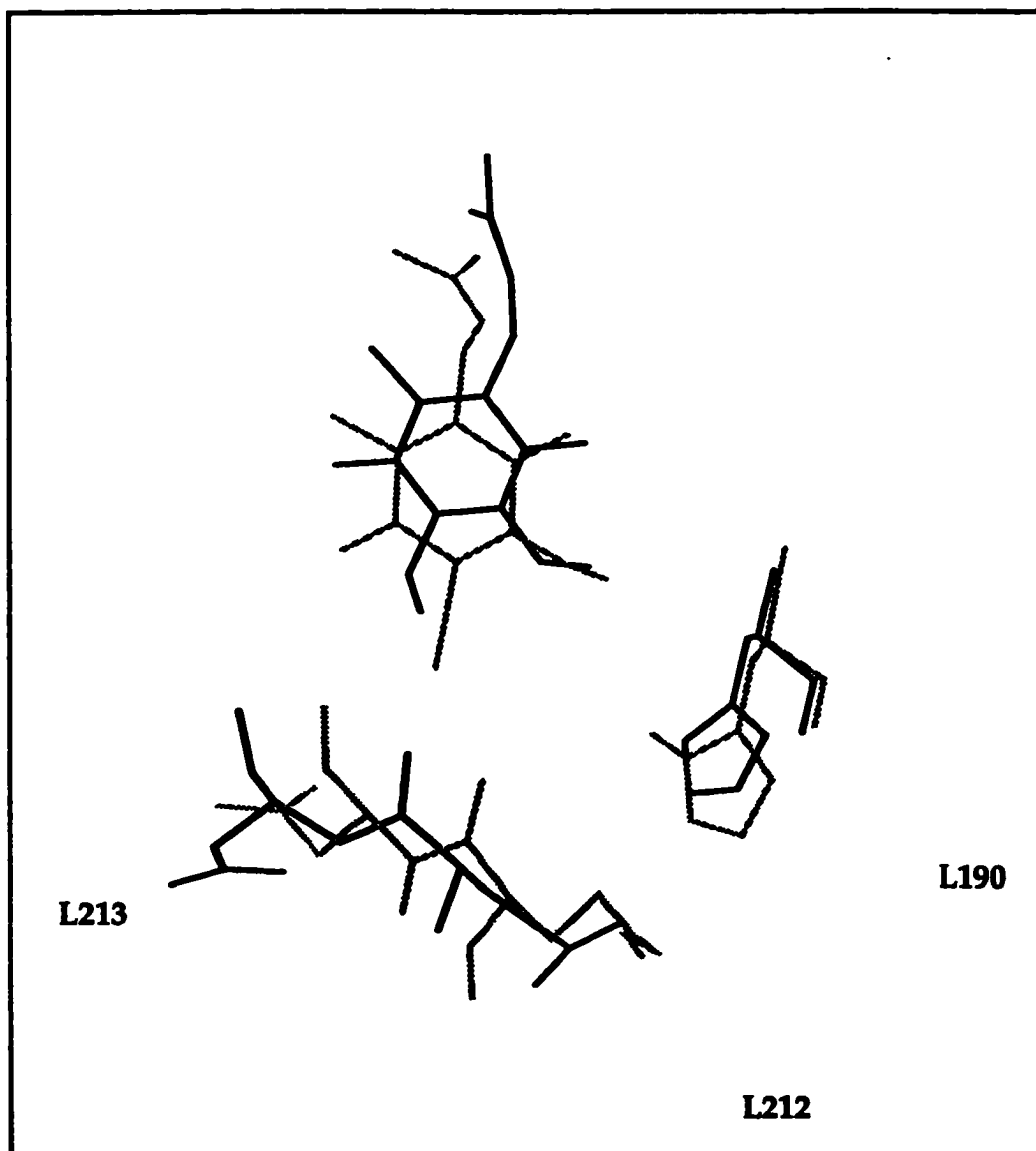


Figure 5-3. A Comparison of the Calculated Average Position of Neutral Q_B in the GLH/ASP Protonation State (grey) with an Experimentally²¹ Determined Position (black). This is the closest match between the predicted and the experimentally observed location.

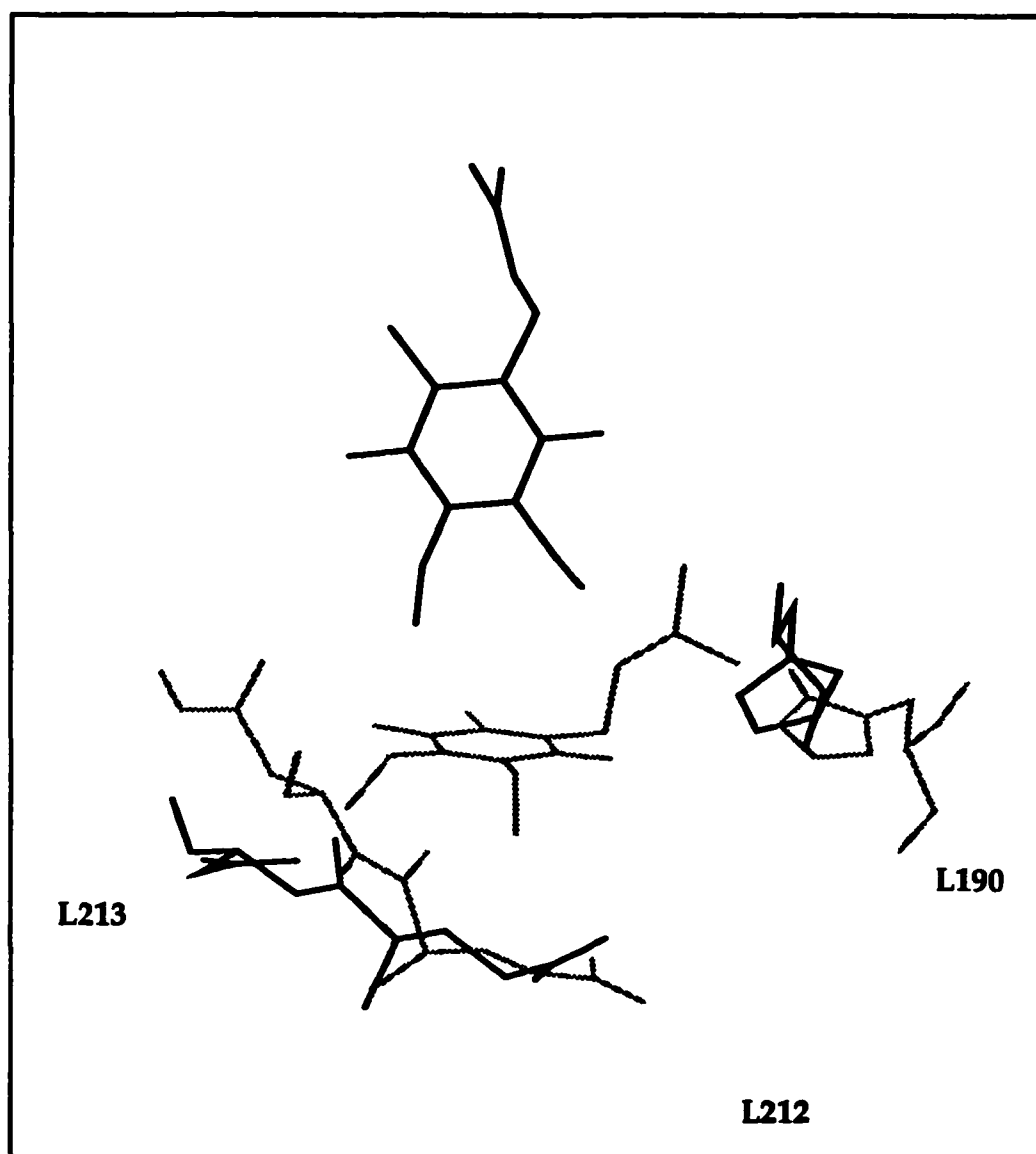


Figure 5-4. A Comparison of the Calculated Average Position of Neutral Q₈ in the GLU/ASH Protonation State (grey) with an Experimentally²¹ Determined Position (black). Clearly, this is not a good match, implying that this protonation state is not likely.

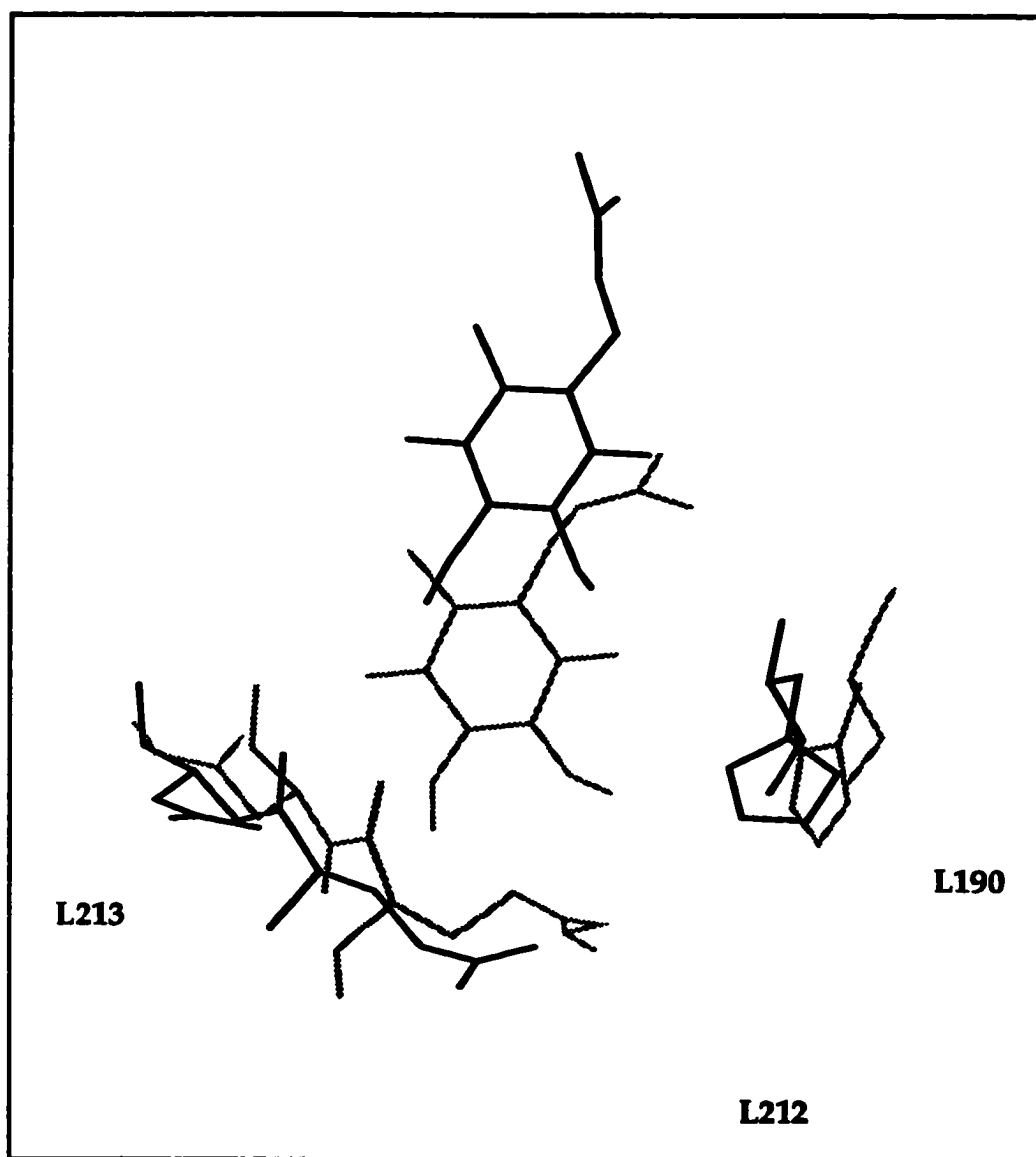


Figure 5-5. A Comparison of the Calculated Average Position of Neutral Q_B in the GLH/ASH Protonation State (grey) with an Experimentally²¹ Determined Position (black). In this protonation state, the quinone is very near the observed "activated" position.

in the ionized state.⁶⁵ The binding site for ubiquinone in the GLH/ASH state, however, corresponds well with four of the five previous X-ray structures (Figure 5-1) and also with the secondary, or $Q_B^{\bullet-}$, binding site reported by Stowell et al. These predicted binding sites therefore support the theory presented here that the differences in the location of Q_B in *Rb. sphaeroides* reaction centers in different X-ray structures are very likely a result of differing protonation states of nearby amino acids, specifically GLU L212 and ASP L213, and not simply because of loose binding of Q_B and/or structural disorder.

5.3.2 Radical Anion $Q_B^{\bullet-}$ Binding

After excitation and electron transfer to Q_B , the radical anion state can exist in the RC for reasonably long times. In the past, the binding site of $Q_B^{\bullet-}$ has been inferred from ESR and vibrational analysis, but recently, Stowell et al. reported an X-ray structure of the RC in the charge-separated state. This group noted that $Q_B^{\bullet-}$ appeared to bind about 5 Å away from the Q_B site, and in an orientation consistent with a 180° twist of the quinone head group about the isoprenoid tail. They speculated that the activation energy for electron transfer was related to the energy necessary to move Q_B from the neutral binding site to the $Q_B^{\bullet-}$ site, where, presumably, electron transfer is more favorable. Table 5-5 and Figures 5-6 through 5-9 provide a comparison of the calculated binding site of $Q_B^{\bullet-}$ in RC with various protonation states of L212 and L213. Although the MD results do not reproduce the 180° twist found in some X-ray structures discussed above, our calculations clearly demonstrate that the only protonation state which satisfactorily reproduces the experimentally determined binding site of $Q_B^{\bullet-}$ is GLH/ASH.

Table 5-5. Calculated Distances (Å) of Ubisemiquinone Radical Anion ($Q_B^{\bullet-}$) Carbonyl Oxygens to Selected Potential Hydrogen Bond Donors for Different Protonation States of GLU L212 and ASP L213 Compared to X-ray Results.

Atoms	Protein Residue	X-ray ^{21,a}	GLU/ASP	GLH/ASP	GLU/ASH	GLH/ASH
O1-N	L182	14.44	12.72	10.20	10.11	14.14
O1-N	L185	11.29	11.19	7.77	7.75	11.00
O1-N	L186	8.76	8.78	4.99	4.91	6.38
O1-N	L187	7.80	10.28	5.75	5.64	7.68
O1-N	L189	6.60	8.97	5.21	5.35	6.44
O1-N	L190	3.89	8.88	4.36	4.61	3.82
O1-ND1	L190	2.81	10.88	6.59	6.82	3.28
O1-OE1	L212	6.67	13.19	10.73	9.93	6.94
O4-ND1	L190	8.13	6.10	7.49	9.11	7.28
O4-OD1	L213	5.29	10.00	5.83	7.98	6.51
O4-N	L216	6.26	7.38	7.62	7.47	6.80
O4-N	L220	9.31	9.70	8.75	7.77	9.76
O4-N	L221	9.31	11.19	8.80	7.17	9.16
O4-N	L222	7.58	11.01	7.05	5.46	7.36
O4-OG	L223	3.21	13.66	4.24	4.55	4.79
O4-N	L223	4.80	11.46	4.68	3.60	4.82
O4-N	L224	2.96	9.98	3.11	2.86	2.92
O4-N	L229	6.29	8.41	5.41	6.62	5.88
Avg. Abs. Difference from X-ray		-	3.75	1.75	2.35	0.66

^a Taken from reference 21 except that the carbonyl oxygen distances are switched for comparison since calculations do not reproduce the 180° quinone rotation about the isoprenyl chain.

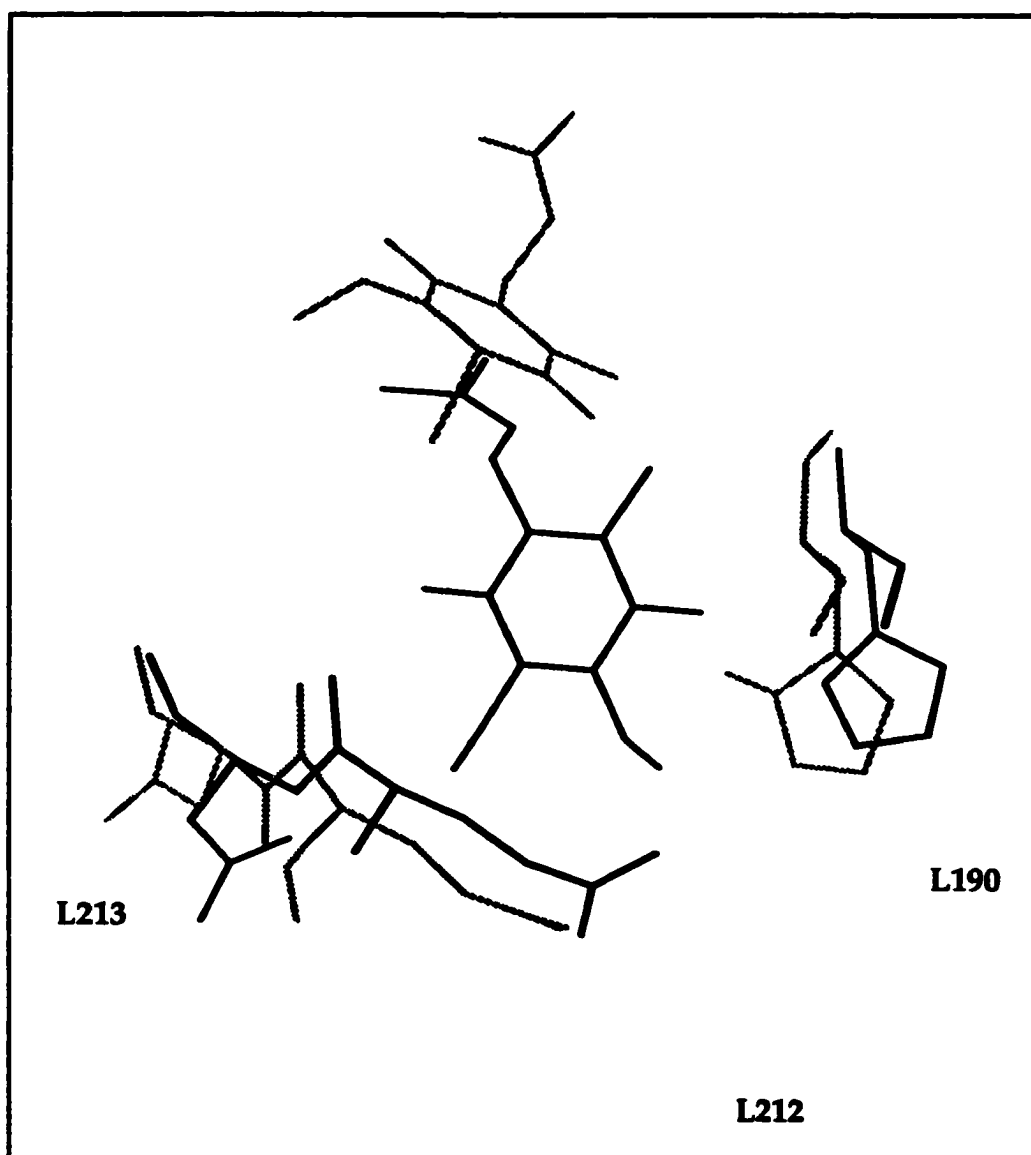


Figure 5-6. A Comparison of the Calculated Average Position of the Radical Anion $Q_B^{\bullet-}$ in the GLU/ASP Protonation State (grey) with an Experimentally²¹ Determined Position (black). The predicted position is quite far from the experimentally observed location.

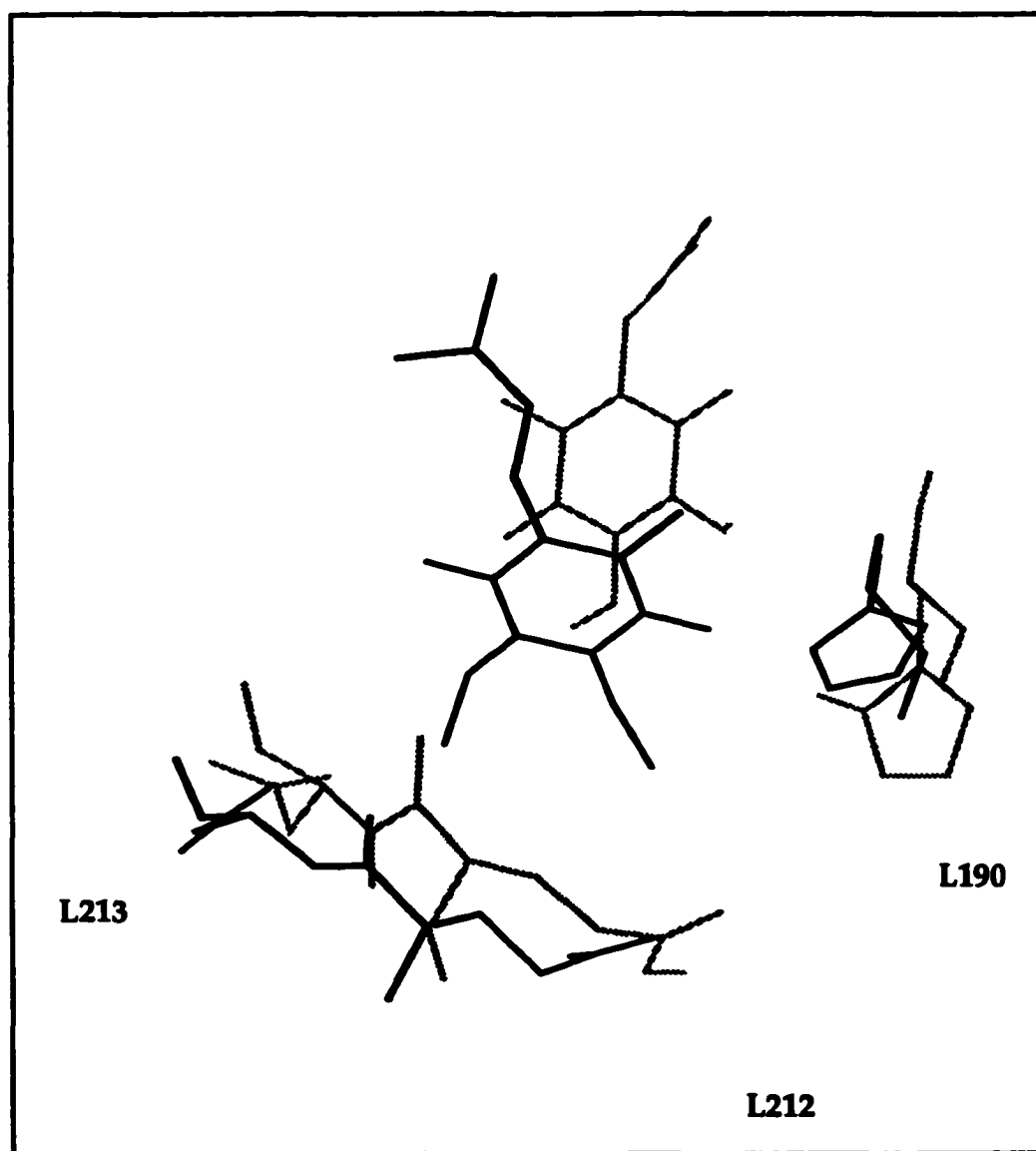


Figure 5-7. A Comparison of the Calculated Average Position of the Radical Anion $Q_B^{\bullet-}$ in the GLH/ASP Protonation State (grey) with an Experimentally²¹ Determined Position (black). Again, this protonation state does not appear to match well with experiment.

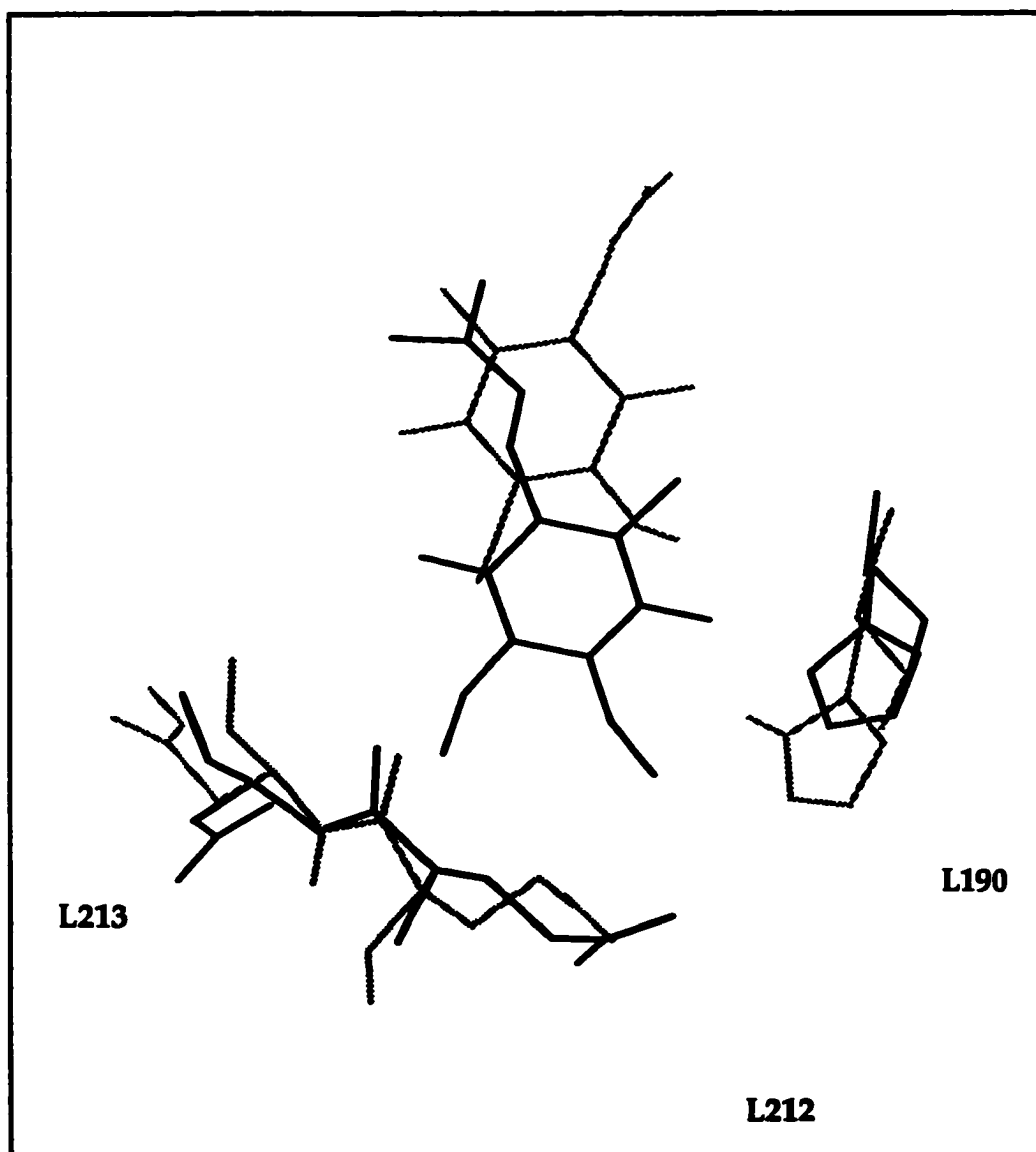


Figure 5-8. A Comparison of the Calculated Average Position of the Radical Anion $Q_B^{\bullet-}$ in the GLU/ASH Protonation State (grey) with an Experimentally²¹ Determined Position (black). Another case where the bad match tends to rule out this protonation state.

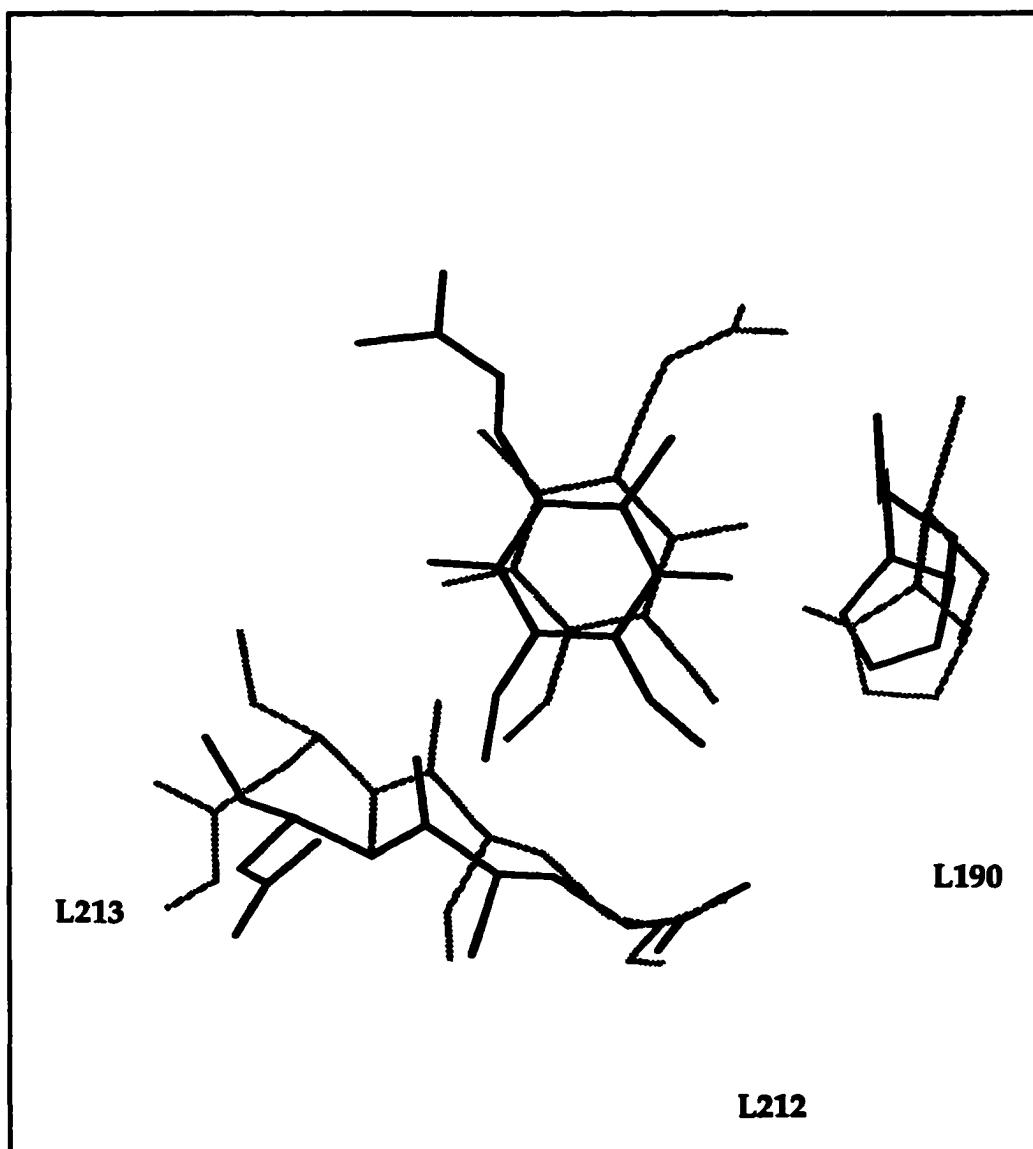


Figure 5-9. A Comparison of the Calculated Average Position of the Radical Anion $Q_B^{\bullet-}$ in the GLH/ASH Protonation State (grey) with an Experimentally²¹ Determined Position (black). The excellent match indicates the likely protonation state for $Q_B^{\bullet-}$ formation.

5.3.3 Interaction Energies

Tables 5-6 and 5-7 report calculated pairwise interaction energies of Q_B and $Q_B^{\bullet-}$ with selected nearby protein residues. These energies are pair energies only for qualitative comparison, and therefore do not include entropic or correlation effects. For neutral ubiquinone, one of the strongest interactions in all amino acid protonation states is with PHE L216, whose aromatic ring side chain is nearby and roughly parallel to the quinone head group. Next is TYR L222, whose interaction with Q_B is much more favorable if one or the other of L212 or L213 is protonated. GLU L212 and ASP L213 interactions with Q_B vary depending on the protonation states, and with both residues ionized, the interaction with ASP L212 is actually slightly unfavorable. The interaction of HIS L190 with Q_B becomes more favorable as the amino acid protonation state increases, consistent with the quinone moving closer as described in Table 5-4. The most unfavorable interaction is with the non-heme iron in the GLU/ASP protonation state at about +1 kcal/mol, but this interaction is at least slightly favorable in all other protonation states.

In Table 5-7, it is quite clear that the nearby ionized amino acids have a very unfavorable (about +30 kcal/mol) interaction with the negatively charged $Q_B^{\bullet-}$. This unfavorable association is offset somewhat by the favorable interaction with the non-heme iron. As protonation increases, so does the favorable interaction with HIS L190 and SER L223, consistent with the formation of strong hydrogen bonds. Both of these residues have been found experimentally to be important in facilitating electron transfer to Q_B . It may be that it is not the movement of Q_B which promotes electron transfer, but that it is instead the protonation of L213, and the subsequent removal of an unfavorable interaction

Table 5-6. The Interaction Energy (kcal/mol) of the Secondary Ubiquinone, Q_B, in the Neutral State with Nearby Protein Residues for Different Protonation States of GLU L212 and ASP L213.

		GLU/ASP	GLH/ASP	GLU/ASH	GLH/ASH
ALA	L186	-3.450	-0.982	-1.524	-1.623
LEU	L189	-3.855	-5.561	-3.389	-1.874
HID	L190	-0.147	-1.868	-2.584	-2.968
LEU	L193	-0.828	-0.696	-2.380	-2.237
GLU	L212	-2.991	-0.442	-5.396	-1.360
ASP	L213	0.130	-4.257	-3.040	-0.942
PHE	L216	-5.788	-6.255	-7.494	-8.843
TYR	L222	-0.409	-7.765	-7.545	-4.217
SER	L223	-3.346	-5.278	-1.784	-2.583
ILE	L224	-0.939	-2.186	-5.651	-5.901
GLY	L225	-0.628	-0.107	-4.452	-3.501
ILE	L229	-1.446	-3.267	-4.952	-5.421
LEU	L232	-0.852	-2.168	-2.192	-4.283
Iron		1.070	-0.059	-3.332	-0.602
Everything Else		-63.620	-36.424	-48.606	-49.539
Total		-87.099	-77.055	-104.321	-95.894

Table 5-7. The Interaction Energy (kcal/mol) of the Secondary Ubiquinone, $Q_B^{\bullet-}$, in the Radical Anion State with Nearby Protein Residues for Different Protonation States of GLU L212 and ASP L213.

		GLU/ASP	GLH/ASP	GLU/ASH	GLH/ASH
ALA	L186	0.162	-1.299	-2.238	-1.177
LEU	L189	-7.615	-8.172	-7.346	-4.419
HID	L190	-6.157	-13.498	-10.455	-21.083
LEU	L193	-4.429	-3.892	-3.280	-6.553
GLU	L212	30.220	-0.141	33.175	-2.106
ASP	L213	30.342	39.262	0.732	0.290
PHE	L216	-6.324	-7.851	-7.263	-6.746
TYR	L222	0.625	0.047	-2.053	0.012
SER	L223	-0.750	-7.355	-13.150	-11.486
ILE	L224	-1.638	-4.495	-7.878	-9.655
GLY	L225	1.728	4.588	3.220	-2.493
ILE	L229	-1.820	-6.492	-5.018	-6.229
LEU	L232	-2.896	-4.072	-4.942	-3.054
Iron		-22.818	-28.469	-25.855	-35.587
Everything Else		-169.632	-72.601	-64.597	-113.238
Total		160.910	-114.467	-116.948	-222.792

between two nearby negative charges ($Q_B^{\bullet-}$ and ionized ASP L213), which makes electron transfer more favorable, and that the movement of Q_B from one site to another is merely a side effect.

5.4 Conclusions

Experimental X-ray structures have revealed different binding sites for the secondary quinone, Q_B , in the reaction center of *Rb. sphaeroides*. Recently, Stowell et al.²¹ reported an X-ray structure determination of the RC in the charge-separated state, providing an unprecedented look at the binding of the radical anion, $Q_B^{\bullet-}$. They noted that the $Q_B^{\bullet-}$ binding site was about 5 Å away from the neutral site, identified in the same study and speculated that the movement of the neutral quinone between these two sites provided for an “activated” structure when the neutral quinone moved to the $Q_B^{\bullet-}$ site, thus facilitating electron transfer. Others have noted the important effect that the nearby amino acid groups GLU L212 and ASP L213 can have on electron transfer. Both of these residues can exist in ionized and protonated forms. We have presented the first molecular dynamics calculations to study the binding of Q_B , and the results show that the different binding sites observed experimentally (speculated to be caused by loose binding or partial occupation of the Q_B site³⁶) may be a direct result of the protonation states of these nearby amino acids. We showed that the neutral binding site identified by Stowell et al., which is also consistent with the structure reported by Ermler et al.,²⁵ is most consistent with either the GLU/ASP or GLH/ASP protonation states, and previous experimental evidence supports the latter state.⁶⁵ We also demonstrated that the Q_B binding site identified in other X-

ray structures and the $Q_B^{\bullet-}$ bind site described by Stowell et al. is only consistent with the GLH/ASH protonation state. Our calculations show that if both L212 and L213 are protonated, then the binding sites of Q_B and $Q_B^{\bullet-}$ are virtually the same, so it is possible that Stowell et al. report two binding sites for Q_B which differ because of differing protonation states of L213. Because our calculations were started with $Q_B^{\bullet-}$ approximately in the neutral, “inactivated” site (as described by Stowell et al.) and no outside forces were imposed, $Q_B^{\bullet-}$ moved to its equilibrium binding site spontaneously, indicating that, if the simulation is an accurate representation of the reaction center, any energy barrier for the movement is no larger than the thermal energy present at 300K, or about 0.6 kcal/mol. This is significantly lower than the 15 kcal/mol activation energy observed experimentally for electron transfer to Q_B . We therefore speculate that the movement of Q_B from the “neutral” to the “anion” site is not the process responsible for the activation energy, rather that the movement is the product of a change in protonation state of one or both of the nearby amino acids, most likely the protonation of ASP L213. Stowell et al. note that their theory is consistent with the fact that electron transfer is not dependent on the reduction potential of the quinone at Q_B . However, it is also known that the kinetics of electron transfer are highly pH dependent. Therefore the activation energy may be due to the electrostatic differences arising during the protonation of ASP L213 (and the associated protein conformational changes), which then results in the movement of Q_B to a binding site more favorable for electron transfer. It is also possible that the reason for the apparent favorable nature of one Q_B binding site over the other for the first electron transfer is due not so much to the site itself, but because of the unfavorable electrostatic interaction between a negatively charged quinone and a negatively charged,

ionized ASP L213 residue which must be protonated to stabilize $Q_B^{\cdot-}$.⁹⁵ Nabedryk et al.³⁴ concluded from FTIR data that ASP L213 does not change protonation state upon formation of $Q_B^{\cdot-}$, which is consistent with our suggestion that L213 must be protonated *before* electron transfer can occur.

However, we must also note that our calculations did not reproduce the observed 180° twist of the quinone about the isoprenyl tail. Therefore our theory that Q_B movement occurs without a significant activation energy due to a change in protonation state of ASP L213 is based on the assumption that this rotation is either not real, instead arising from uncertainties in the X-ray structures available (which may be doubtful since five structures, including Stowell et al.'s, agree on the orientation of the quinone in the “anion” binding site, while the two structures that place the quinone in the other binding site show opposite orientation), or that the energy necessary to perform the rotation is not significant relative to the energy associated with protonating ASP L213. Our calculations do not address the effects of amino acid protonation states on the second electron transfer to Q_B , a process which is coupled to proton transfer to the quinone, presumably from a nearby amino acid. Such a future study will likely require the inclusion of quantum effects as well as molecular dynamics. Further study is clearly warranted to determine unambiguously the origin of the activation energy of the first Q_A to Q_B electron transfer event in bacterial photosynthesis.

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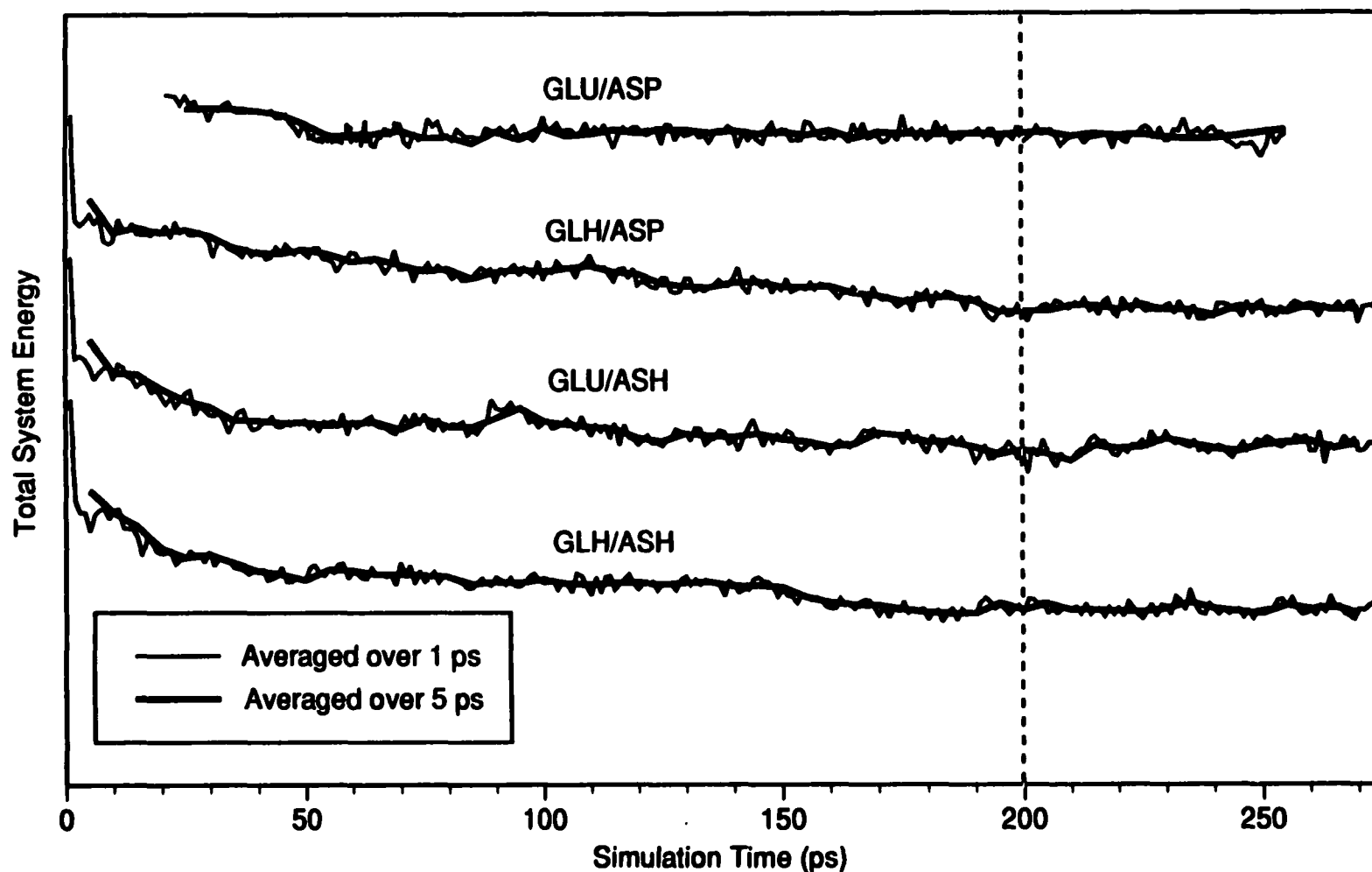


Figure S5-1. Changes in the Total System Energy of Each Simulated Amino Acid Protonation State Versus Simulation Time for the QA-/QB Systems. To the right of the dotted line is the time during which structural "snapshots" were taken every 0.05 ps for average structure determination. Because the total system energies remain relatively constant in this area, the systems are assumed to be at equilibrium. 75 ps of data was used to determine each average structure (some energy data for the GLU/ASP state was lost).

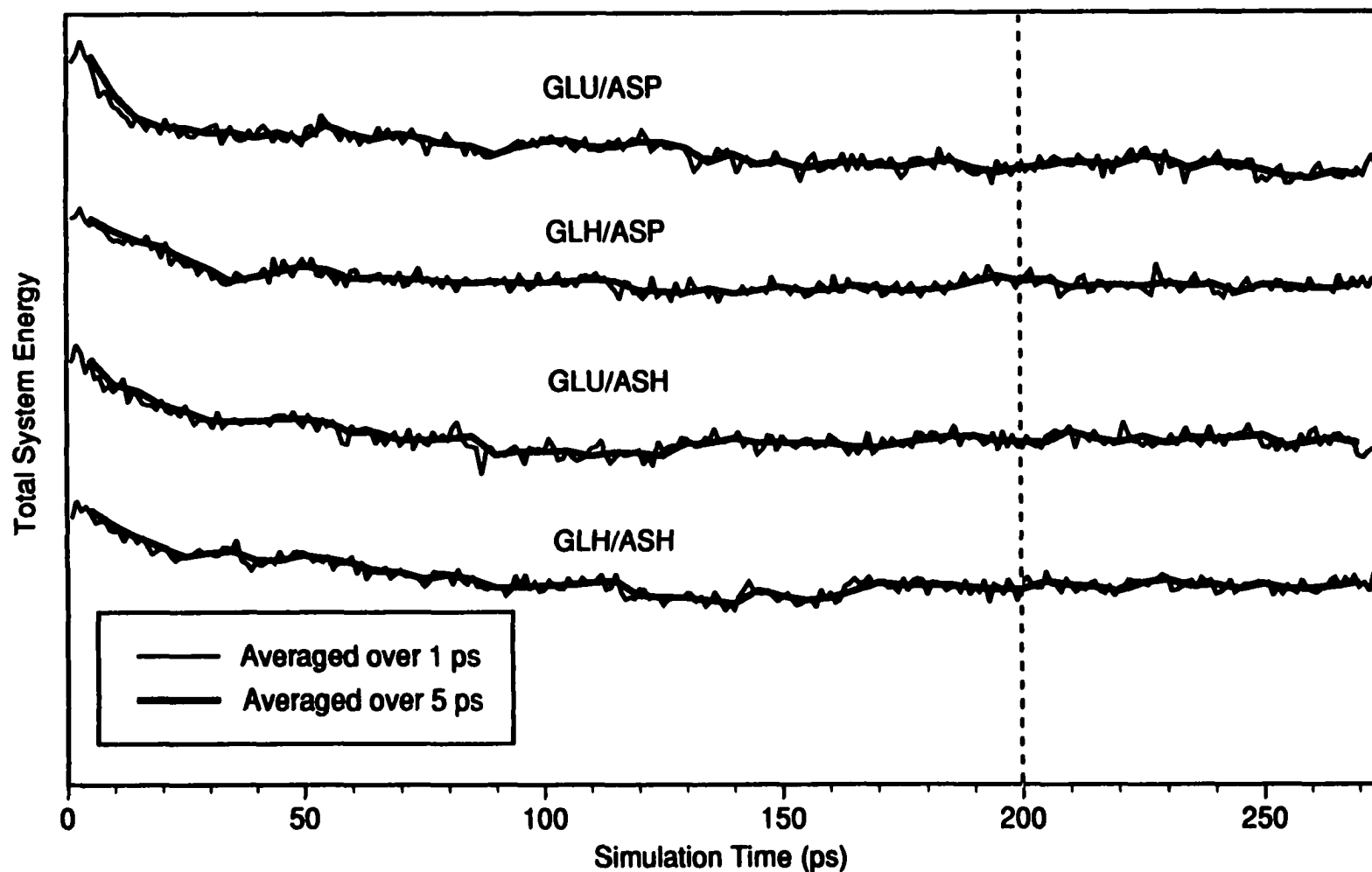


Figure S5-2. Changes in the Total System Energy of Each Simulated Amino Acid Protonation State Versus Simulation Time for the QA/QB- Systems. To the right of the dotted line is the time during which structural "snapshots" were taken every 0.05 ps for average structure determination. Because the total system energies remain relatively constant in this area, the systems are assumed to be at equilibrium. 75 ps of data was used to determine each average structure.

CHAPTER 6

Conclusions and Future Work

Reaction centers from certain purple bacteria provide model systems for studying the fundamental chemical processes involved in photosynthesis. Electron transfer to *para*-quinones is an important part of photosynthesis, and the study of quinones as electron acceptors is currently a very active field of research. In particular, there is widespread interest in the short lived intermediates formed when quinones accept electrons (and protons), as well as how these various species interact with the protein environment of reaction centers to facilitate electron transfer. Especially interesting is the ability of the reaction center of *Rhodobacter sphaeroides* to attenuate the function of the native ubiquinone so that it can react differently depending on whether it is at the primary, Q_A , site or the secondary, Q_B , site. Spectroscopic methods have long been used to study quinones and their reduced and protonated species *in vivo* and *in vitro*, but the results can be difficult to interpret and are often complicated by interference from the chromophores or the protein itself. More recently, atomic resolution structural data has been made available for bacterial reaction centers, including one which reportedly identifies the binding site of the secondary quinone in its radical anion form. Questions still remain to be answered about the structure, spectra, and behavior of quinones and quinone intermediates involved in photosynthesis. In this study, work was presented which seeks to address some aspects of these questions using modern computational chemistry techniques.

First, it was shown that the relatively new hybrid Hartree-Fock/density functional (HF/DF) method Becke3LYP (B3LYP) in combination with a modest 6-31G(d) basis set can accurately and efficiently reproduce the geometries, vibrational spectra, and spin properties of a variety of 1,4-naphthoquinone derivatives, including vitamin K, and their radical anions. It was demonstrated that B3LYP was consistently closer to experimental results than pure density functional methods. Spin densities and proton hyperfine coupling constants were shown to be well reproduced with variety of basis sets, while heavy atom hyperfine coupling constants were more sensitive to basis set size, possibly requiring larger basis sets which have been augmented in the core region for reliable results. Additionally, the first full description of the vibrational spectra of several 1,4-naphthoquinone radical anion derivatives were provided to aid the interpretation of experimental results.

When comparing the vibrational spectra between a neutral molecule and its radical anion, it is important to be able to predict how certain modes will change upon reduction. Identifying which modes best match between similar species has, in the past, been largely a qualitative decision. To remedy this situation, the vibrational projection analysis (ViPA) method was developed and presented as a quantitative and consistent means of comparing the normal modes of vibration not only of a neutral molecule and its radical anion, but any two molecules which share a similar substructure. ViPA has been shown to have several advantages over other mode comparison methods, and it was used extensively throughout this work to compare the vibrational modes of quinones and their derivatives.

In addition, a detailed analyses of the vibrational spectra of a variety of methylated *para*-quinones and many of their reduced, protonated, and hydrogen

bonded forms has been presented. The effect of protonation and hydrogen bonding on quinones is a complex issue important to the interpretation of experimental spectra. The first complete analysis of the vibrational spectra of all the methyl-substituted derivatives of 1,4-benzoquinone and their radical anions was presented and compared to experimental data where available. Also, the first complete descriptions of the vibrational spectra of neutral radical, dianionic, and doubly protonated (quinol) forms of 1,4-benzoquinone, 2,3,5-trimethyl-1,4-quinone, and 1,4-naphthoquinone were presented. It was shown that the B3LYP/6-31G(d) method is often at least as good as more complicated methods at reproducing the limited experimental spectroscopic features, except in the case of the neutral radical forms. Also for the first time, a complete analysis of the effect of hydrogen bonding on the vibrational spectra of 1,4-benzoquinone was presented and discussed relative to experimental data. Most of the changes in frequencies noted from previous experiments were reproduced by the B3LYP/6-31G(d) method, and certain vibrational modes were suggested as “markers” for use in determining the protonation or hydrogen bonded state of quinones observed experimentally.

Next, it was demonstrated that a combination of HF/DF methods and molecular dynamics calculations can provide exceptionally accurate thermodynamic data for biologically important quinones. It was shown that the B3LYP/6-311G(d,p) method can be used to predict one-electron adiabatic electron affinities for a variety of methylated and fused-ring quinones within 0.05 eV of experimental values. Combining this data with aqueous free energy differences between a quinone in its neutral and radical anionic forms calculated with molecular dynamics was shown to provide very good one-electron reduction

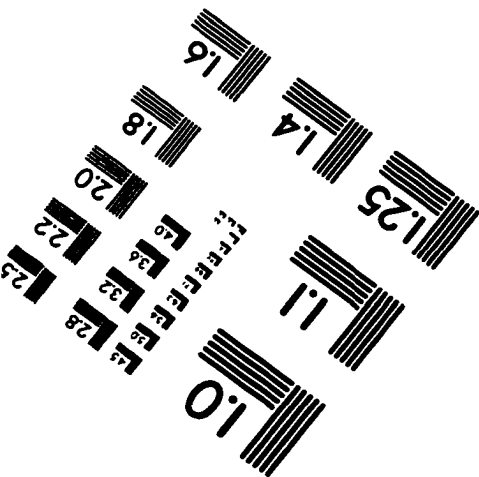
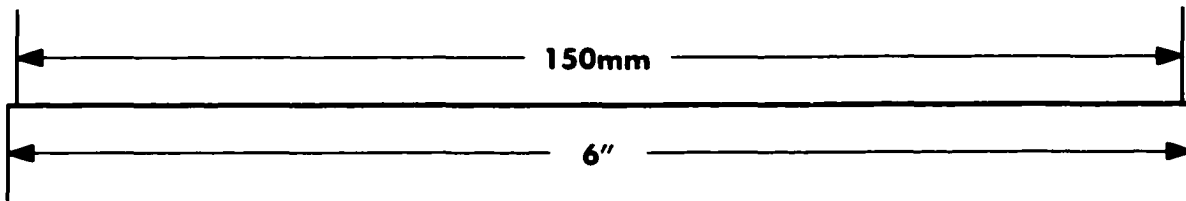
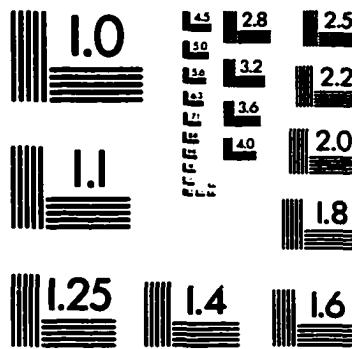
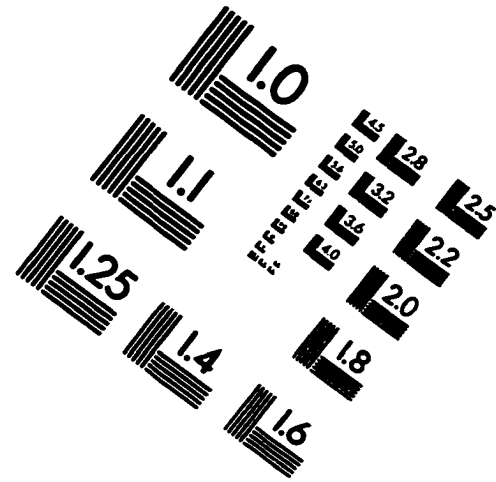
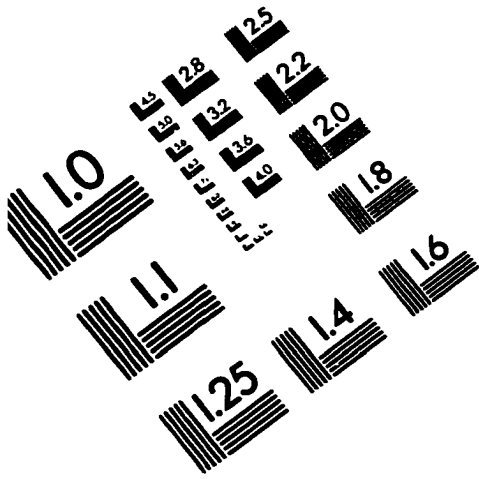
potentials for quinones, usually within about 0.2 eV of experimentally determined values. In the case of plastoquinone and ubiquinone, both of which are vital in photosynthetic processes, calculated values for reduction potentials were within about 0.05 eV of experiment. The ability to predict gas phase electron affinities and to develop molecular dynamics force fields which reliably reproduce reduction potentials are both very important to modeling the process of photosynthesis itself.

Lastly, molecular dynamics simulations of the photosynthetic reaction center of *Rb. sphaeroides* were presented to describe the binding of the secondary ubiquinone (Q_B) in both the neutral and radical anionic forms. The data presented demonstrate the importance of the nearby protein residues GLU L212 and ASP L213 in determining the binding site of Q_B . Both of these amino acids can exist in protonated and ionized forms. It was demonstrated that the differences in the binding sites of Q_B and $Q_B^{\cdot-}$ observed experimentally by X-ray crystallography may be explained by differences in the protonation states of L212 and L213. The predicted binding of Q_B was closest to experiment when L212 was protonated and L213 was ionized. The observed binding site of $Q_B^{\cdot-}$ was reproduced only when both protein residues were protonated. It was then suggested that instead of the movement of the secondary quinone from one site to another being responsible for the experimentally observed activation energy of electron transfer, the movement itself is a product of the protonation of ASP L213. It was speculated that the movement of Q_B upon protonation of L213 is spontaneous at room temperature, and that the processes involved in transferring a proton to L213 may be the cause of the observed activation energy. In the GLH/ASH protonation state, electron transfer may be the rate-limiting step. Additionally, it is also possible that the protonation state may remain constant (GLH/ASP) and the measured activation

energy would then be due to electron transfer or short-range migration of the secondary quinone. The molecular dynamics calculations did not reproduce an experimentally observed rotation of Q_B about its isoprenoid tail during movement from one site to another. This rotation could be energetically important in the confined binding site of Q_B , and should be investigated further, as it could account for part or all of the observed activation energy.

Overall, this study has demonstrated the ability to successfully model a variety of properties of the quinones vital to photosynthesis. The next steps which could be taken might first involve using potential of mean force calculations to move Q_B from one binding site to another, and also to predict the energy needed to rotate the quinone head group. Next, it would be desirable to demonstrate the ability to provide results consistent with experiments that still offer new insights into existing questions. Quantum calculations could be performed on any of a variety of possible substitutes for the native quinone, and their effect on electron transfer rates could be predicted and suggestions could be made about which experimentally studies may yield the most insight into the chemical processes involved. Models of other reaction centers, including those that involve site-directed mutagenesis, might be performed to further study the effect of the protein environment on electron transfer. In conclusion, many possible avenues for the investigation of photosynthesis by theoretical methods are now open since the techniques described in this work have been shown to provide consistent and accurate results.

IMAGE EVALUATION TEST TARGET (QA-3)



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