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New Insights into Quinine–DNA Binding Using Raman Spectroscopy and Molecular Dynamics Simulations

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Abstract

Quinine's ability to bind DNA and potentially inhibit transcription and translation has been examined as a mode of action for its antimalarial activity. UV absorption and fluorescence-based studies have lacked the chemical specificity to develop an unambiguous molecular-level picture of the binding interaction. To address this, we use Raman spectroscopy and molecular dynamics (MD) to investigate quinine–DNA interactions. We demonstrate that quinine's strongest Raman band in the fingerprint region, which derives from a symmetric stretching mode of the quinoline ring, is highly sensitive to the local chemical environment and pH. The frequency shifts observed for this mode in solvents of varying polarity can be explained in terms of the Stark effect using a simple Onsager solvation model, indicating that the vibration reports on the local electrostatic environment. However, specific chemical interactions between the quinoline ring and its environment, such as hydrogen bonding and π -stacking, perturb the frequency of this mode in a more complicated but predictable manner. We use this vibration as a spectroscopic probe to investigate the binding interaction between quinine and DNA. We find that, when the quinoline ring is protonated, quinine weakly intercalates into DNA by forming π -stacking interactions with the base pairs. The Raman spectra indicate that quinine can intercalate into DNA with a ratio reaching up to roughly one molecule per 25 base pairs. Our results are confirmed by MD simulations, which also show that the quinoline ring adopts a t-shaped π -stacking geometry with the DNA base pairs, whereas the quinuclidine head group weakly interacts with the phosphate backbone in the minor groove. We expect that the spectral correlations determined here will enable future studies to probe quinine's antimalarial activities, such as disrupting hemozoin

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jpcb.8b05795](https://doi.org/10.1021/acs.jpcb.8b05795). Methodological descriptions of the fluorescence measurements, DFT calculations, normal mode analysis, MD simulations, and computational analysis; scripts and input files used for computations and data analysis; tables showing the band assignments of un-, mono-, and diprotonated quinine; Raman spectra showing the effects of aggregation and high-ionic-strength solutions on the quinoline ring symmetric stretching mode of quinine, rheological data, and fluorescence spectra of quinine–DNA binding (PDF)

Notes

The authors declare no competing financial interest.

biocrystallization, which is hypothesized to be, among other things, one of its primary modes of action against *Plasmodium* parasites.

Graphical Abstract

INTRODUCTION

Quinine is a cinchona alkaloid that has been used as an antimalarial drug for hundreds of years.¹ In modern times, the quinine derivatives chloroquine and mefloquine are the drugs of choice to treat malaria. However, the emergence of drug-resistant malarial strains has prompted renewed interest in quinine, which surprisingly remains an effective alternative to its more potent counterparts against all four *Plasmodium* spp. parasites that cause malaria.² Despite the continued importance of quinine as a broad-spectrum, low-cost antimalarial drug, its principal mechanism of action remains unclear. This critical shortcoming needs to be addressed to continue the development of effective antimalarial drugs.

One problem in establishing quinine's primary mechanism of action stems from the fact that it exerts multiple toxic effects on both parasitic and mammalian host cells.^{3–5} Three main hypotheses have been proposed for quinine's antimalarial activity. One hypothesis is that quinine, which is thought to be a lysosomotropic agent,^{6,7} alkalinizes acidic food vacuoles of malarial parasites, disrupting hemoglobin metabolism. Another hypothesis proposes that quinine binds hematin and disrupts hemozoin biocrystallization,^{8,9} effectively killing the parasites with their own metabolic waste. A third hypothesis is that quinine disrupts transcription and replication by intercalating into DNA, including that of the malarial parasites.¹⁰

Understanding the DNA binding abilities of quinine and its derivatives has garnered renewed interest in developing anticancer drugs.¹¹ The interaction of quinine and its derivatives with DNA has been studied since the late 1940s. Early studies relied on UV absorption and viscometric measurements and indicated that quinine, chloroquine, and mefloquine bind DNA weakly, at high concentrations.^{10,12–18} Most of the structural insights formulated in these studies come primarily from the construction of crude CPK spacefilling models. At best, these models suggest that quinine and chloroquine can partially intercalate into the DNA duplex to enable π -stacking interactions with the base pairs.¹⁸ A later study, by Lai et al.,¹⁹ used fluorescence spectroscopy to probe the binding interaction. However, the authors

could only conclude that hydrophobic forces were important in quinine—DNA interactions and that the binding is spontaneous due to the complex dependence of quinine's fluorescence intensity to self-quenching, chloride ions, and DNA. Importantly, these studies have failed to provide a molecular-level picture of the quinine—DNA binding geometry.

The lack of a more detailed molecular-level picture motivates the need to re-examine quinine—DNA binding with more incisive structural characterization and computational methods. Vibrational spectroscopy is an especially powerful tool for investigating the local structure and chemical environment of molecules. Infrared and Raman spectroscopic probes, for example, monitor the local dielectric and hydrogen bonding environments of proteins,^{20–22} DNA, and amino acid side chains.^{23–26}

Previous studies by Weselucha-Birczyńska and Nakamoto^{27,28} attempted to use Raman spectroscopy to investigate the binding of the quinine derivative, cinchonine, to DNA. These studies, however, did not sufficiently develop a molecular-level picture of the binding interaction. This occurred, in part, because the degree to which the quinoline ring modes of cinchonine were environmentally sensitive was not yet known. Therefore, to capitalize on the sensitivity of infrared and Raman probes, the origins of their vibrational shifts in different environments must be comprehensively examined.

The sensitivity of vibrational probes stems from frequency shifts that occur in different chemical environments due to either bond polarization changes or changes in the local electric field, which perturb the vibrational energy transitions.²⁹ In the case of bond polarization, the frequency shifts arise from changes in the bond force constants. The bond force constants change because more (less) polar environments stabilize resonance structures with weaker (stronger) bonds. This effect changes the relative displacement of the ground-state energy potential along the vibrational normal coordinate relative to the first excited state potential, as well as alters its anharmonicity.²⁹

In contrast, the frequency sensitivity to local electric field changes is due to the vibrational Stark effect. This effect arises from the fact that the local environment exerts an electric field onto a chromophore, altering its vibrational transition energy in a manner that depends on the difference in the dipole moments of the ground and first excited vibrational states. Recent work by the Boxer group^{30–34} and others^{35–39} show that the frequency variation of some vibrational probes, such as carbonyls,^{33,34,37} are well described by the vibrational Stark effect. However, the solvatochromic trends of other probes, such as nitriles,^{38,40–43} cannot be understood solely on the basis of electrostatics due to substantial quantum mechanical effects on the vibrational frequencies.

In this study, we comprehensively investigate the vibrational solvatochromism of quinine in different aprotic and nonaromatic solvents of varying polarity to discern whether any of its modes are sensitive to their local electrostatic environment. Using both Onsager's theory⁴⁴ and atomistic molecular dynamics (MD) simulations, we find that the solvatochromic shifts of the quinoline ring symmetric stretching mode are well described by the vibrational Stark effect, indicating that this vibration reports on the local electrostatic environment. We also show that solvents capable of forming hydrogen bonding and π -stacking interactions with

quinine shift the frequency of this mode in a manner that is difficult to understand in terms of a simple, linear Stark effect. Despite this, hydrogen bonding and π -stacking perturb the frequency of the quinoline ring stretching mode in characteristic manners. We capitalize on the environmental sensitivity of this vibrational mode to probe the binding interaction between quinine and DNA. We complement our Raman measurements with MD simulations and fluorescence spectroscopy, which we use to develop a molecular-level picture of the binding interaction between DNA and quinine.

EXPERIMENTAL SECTION

Materials.

Acetonitrile (99.8% purity), dichloromethane (99.5%), chloroform (99.5%), anisole (99%), methanol (99.8%), quinoline (98%), water (HPLC-grade), hydrochloric acid (HCl, ACS-grade), and calf-thymus DNA (Premium-grade) were purchased from Sigma-Aldrich. Acetone (99.9%), toluene (99.9%), isopropanol (99.9%), and tetrahydrofuran (certified-grade) were purchased from Fisher Chemical. Ethanol (ACS/USP-grade) was purchased from Pharmco-Aaper, and quinine (99% total base, up to 5% dihydroquinine) was purchased from Alfa Aesar. Sodium acetate trihydrate (EMSURE, ACS, ISO, Reag. Ph. Eur.-grade) was obtained from Merck, and glacial acetic acid (ACS-grade) purchased from VWR.

Sample Preparation.

Solutions of quinine in organic solvents for the solvatochromic shift experiments were prepared at concentrations ranging from ca. 5 to 25 mg mL⁻¹, depending on the solubility. For the pH-dependent experiments, quinine solutions were prepared in water at 10 mg mL⁻¹. HCl was used to adjust the acidity of the solutions to pH values ranging from 2.3 to 5.1. The solutions were also prepared with 1% (v/v) acetonitrile, which was used as an internal intensity standard for the Raman spectroscopic measurements. Stock solutions of quinine and calf-thymus DNA were prepared at 6 and 8 mg mL⁻¹, respectively, in 0.1 M sodium acetate buffer (pH 4.3). These stocks were sonicated and used to prepare solutions of quinine (1.5 mg mL⁻¹), DNA (4 mg mL⁻¹), and quinine (1.5 mg mL⁻¹) mixed with DNA (4 mg mL⁻¹) in sodium acetate buffer.

Raman Spectroscopy.

Raman spectra were measured using a 785 nm diode laser purchased from Innovative Photonic Solutions. The beam was directed through a 30/70 beamsplitter (CVI Laser) and focused onto the sample with an infinity-corrected Olympus Ach 10×/0.25 NA objective. Typical laser powers used ranged from 35 to 40 mW at the sample. The scattered light was collected using a 180° backscattering geometry and redirected through the beamsplitter into the spectrometer (Acton SpectraPro 2500i). The light was dispersed in the spectrometer using a 600 gr/mm grating and imaged using a PIXIS CCD camera (Princeton Instruments). The acquisition times and frames collected varied depending on the sample and the concentrations used, but typically ranged between 10 ms and 300 s.

COMPUTATIONAL SECTION

Molecular Dynamics (MD) Simulations.

A detailed description of the computational methods is presented in the Supporting Information (SI). Briefly, MD simulations were performed using NAMD⁴⁵ version 2.12 and force field parameters from CHARMM36⁴⁶/CGenFF⁴⁷ or Amber ff99SB⁴⁸/GAFF.⁴⁹ All simulations were conducted under the NPT ensemble at 300 K and 1 atm. Systems of one quinine molecule were solvated in $40 \times 40 \times 40 \text{ \AA}^3$ periodic boxes of tetrahydrofuran, dimethyl sulfoxide, acetone, acetonitrile, anisole, chloroform, dichloromethane, methanol, toluene, and TIP3P⁵⁰ water. Each of these systems were simulated for 100 ps of equilibration and 2 ns of data production MD. Quinine–DNA systems composed of diprotonated quinine molecules and 11-base pair adenine–thymine (A–T) or cytosine–guanine (CG) DNA duplexes were solvated in $68 \times 68 \times 82 \text{ \AA}^3$ periodic boxes of TIP3P⁵⁰ water. These systems were equilibrated for 400 ps, and data was collected during 40 ns production simulations. The trajectories from the quinine–DNA production simulations were analyzed with VMD,⁵¹ and structures of quinine bound in the minor groove of both the (AT)₁₁ and (CG)₁₁ DNA systems were identified by searching for persistent hydrogen bonding and π -stacking interactions between the molecules. To do this, we defined a hydrogen bond as having a heavy atom donor–acceptor distance of $3.5 \text{ \AA}$ and bond angles of $180 \pm 35^\circ$. We defined plane-edge π -stacking interactions using a distance metric of $5 \text{ \AA}$ between the edge of the nucleobases to the plane of the quinoline ring of quinine, similar to that in the analysis of histidine-adenine stacking by Rutledge et al.⁵² Only the quinine–DNA-bound structures that fit these criteria were extracted from the simulation and analyzed further to quantify the local electric field due to binding. Details regarding the methods used to calculate the local electric fields and binding free energies are discussed in the SI.

RESULTS AND DISCUSSION

Raman Spectrum of Quinine.

The Raman spectrum of quinine in methanol solution, excited within the biologically relevant NIR water window at 785 nm, is shown in Figure 1a. The fingerprint region is complex; however, previous studies have suggested band assignments for quinine^{53,54} and its derivatives^{27,28,54,55} on the basis of density functional theory (DFT) calculations. We used these previous studies and the Raman spectrum of quinoline (Figure 1b) to guide our band assignments of quinine (Figure 1c–h). To more definitively assign the spectrum, we also performed our own DFT frequency calculations using M06-2X/aug-cc-pVDZ level of theory^{56,57} and explicitly calculated the potential energy distribution (PED) of quinine's normal modes, which previous studies had not done (see the SI and Table S1 for details). Overall, our assignments are in good agreement with previous reports that discuss the topic in greater detail.^{28,53,54}

The most intense Raman bands of quinine and quinoline are located at ca. 1370 and 1375 cm^{-1} , respectively. According to our normal mode analysis, the main contributions to the PED of this vibration are stretching motions of the atoms located in the quinoline ring (Table S1 and Figure 1f). We therefore assign the 1370 cm^{-1} band of quinine to a quinoline ring

symmetric stretching mode. This mode, as noted by Frosch et al.,⁵³ exhibits a significant frequency shift going from aqueous solution to solid state. They consequently propose that this band serves as a spectroscopic marker to monitor the local chemical environment of the quinoline ring moiety of quinine. Building upon Frosch et al.'s⁵³ hypothesis, we set out to further investigate the environmental sensitivity of the quinoline ring vibrations of quinine. We discuss the physical origins of the environmental sensitivity of the quinoline ring symmetric stretching mode below.

Environmental Sensitivity of Quinine's Quinoline Ring Symmetric Stretching Mode.

Because many vibrational transitions depend significantly on local changes in the electric field, one method to examine the environmental sensitivity of a probe is through Stark spectroscopy. According to the Stark effect, a vibrational probe that experiences a change in its local electrostatic environment will exhibit a frequency shift ($\Delta\tilde{\nu}$) that is linearly proportional to the difference in the electric field ($\Delta\vec{E}$)³²

$$\Delta\tilde{\nu}(\text{cm}^{-1}) = -\frac{\Delta\vec{\mu}_{\text{probe}}}{hc} \cdot \Delta\vec{E} \quad (1)$$

where h is Planck's constant, c is the speed of light, and $\Delta\vec{\mu}_{\text{probe}}$ is the difference dipole moment between the ground and excited vibrational states. The magnitude of $\Delta\vec{\mu}_{\text{probe}}$ is known as the Stark tuning rate.⁵⁸

Equation 1 specifies that the change in the vibrational frequency can be related to the difference in the electric field between two electrostatic environments. If eq 1 is referenced to vacuum, it can be rearranged to give

$$\tilde{\nu}_i(\text{cm}^{-1}) = -\frac{\Delta\vec{\mu}_{\text{probe}}}{hc} \cdot \vec{E}_i + \tilde{\nu}_{\text{vacuum}} \quad (2)$$

where $\vec{E}_i$ is the electric field felt by the probe in the i th solvent environment and $\tilde{\nu}_{\text{vacuum}}$ is the frequency of the probe in the gas phase. The electric field in vacuum is taken to be 0 MV cm⁻¹.

A simpler method than Stark spectroscopy to investigate the environmental sensitivity of a vibrational probe is to measure its frequency shifts in different solvents that vary in properties such as their polarity, donor number, or acceptor number. The frequency shifts observed in the vibrational probe in different solvents can be correlated to their dielectric constants and other empirical polarity scales. These correlations can aid in formulating Badger–Bauer relationships that map the change in frequency to the interaction enthalpy between the vibrational probe and its local environment.^{20,59}

Although useful, correlations to empirical solvent properties do not easily provide fundamental physical insights into the system's microscopic environment. However,

solvating molecules exert an electric field, called a reaction field, on the solute that depends on the solvent polarity. Therefore, a useful correlation, which provides more intuitive physical insights, is to relate the reaction fields to the frequency shifts observed for a vibrational probe in different solvents of varying polarities. The solvatochromic shifts should correlate linearly with the solvent reaction fields, as described by eq 1, if electrostatics primarily influence the frequency of the vibrational probe. This technique, known as Stark shift spectroscopy, has been popularized in recent years by Boxer and co-workers,^{32,34} who have shown that the solvatochromic shifts of many vibrational probes can surprisingly be described in terms of a simple linear Stark effect.

Inspired by the Boxer group, we measured the Raman spectra of quinine in different solvents of varying polarities and examined the frequency changes that occur in the quinoline ring modes. We estimated the average electric fields that these solvents exert on quinine using the Onsager model,⁴⁴ which assumes the solute of interest occupies a spherical cavity that is embedded in a continuum dielectric that represents the solvent. The magnitude of the solvent's reaction field is calculated using the following equation³²

$$\left| \vec{E}_{\text{Onsager}} \right| = \frac{|\vec{\mu}_0|}{a^3} \left[\frac{2(\epsilon - 1)(n^2 + 2)}{3(2\epsilon + n^2)} \right] \quad (3)$$

The term, ϵ , is the solvent's dielectric constant, whereas n , a , $\vec{\mu}_0$ are the solute's refractive index, cavity radius, and electronic ground-state gas-phase dipole moment, respectively.

For all calculations using eq 3, we used a reference value for the refractive index of quinine ($n = 1.625$).⁶⁰ The term, a^3 ($106 \text{ \AA}^3 \text{ molecule}^{-1}$), was calculated by first finding the cavity volume and then multiplying this value by a factor of $3/4\pi$.³³ The cavity volume was found by dividing quinine's molecular weight ($324.42 \text{ g mol}^{-1}$) by its density (1.21 g mol^{-3}). The gas-phase dipole moment, $\vec{\mu}_0$, was obtained from DFT energy calculations, the magnitude of which was determined to be 3.1679 D . To calculate the solvent reaction fields, we used the following conversion factors:³¹ $1 \text{ D} = 0.03336 \text{ aC \AA}$ and $1 \text{ \AA}^3$ of polarizability volume = $0.01113 \text{ aC \AA}^2 \text{ V}^{-1}$.

We examined the solvatochromic frequency responses (Figure S3) of the quinine modes shown in Figure 1c–h for several aprotic, nonaromatic solvents (dimethyl sulfoxide, acetonitrile, acetone, dichloromethane, tetrahydrofuran, and chloroform). Compared with the other modes, the quinoline ring symmetric stretching vibration displays the largest solvatochromic response (Figure S3d) and the strongest linear correlation with an r^2 coefficient of 0.99. We note that removing the gas-phase data point does not significantly weaken the linear correlation between solvent field and vibrational frequency, with the r^2 value decreasing only to 0.92 when this point is removed. Least-square fitting of the data corresponding to the quinoline ring symmetric stretching mode (Figure 2a,b) to eq 2 yields the following

$$\tilde{\nu} \text{ (cm}^{-1}\text{)} = -0.88 \text{ (cm}^{-1} / \text{MV cm}^{-1}\text{)} \vec{E}_{\text{Onsager}} \text{ (MV cm}^{-1}\text{)} + 1376 \text{ (cm}^{-1}\text{)} \quad (4)$$

The slope in eq 4 corresponds to the apparent Stark tuning rate.³⁴ It is close to typical values measured for nitriles (ca. 0.6–0.7 cm⁻¹/MV cm⁻¹)³² but lower than those measured for carbonyls (ca. >1 cm⁻¹/MV cm⁻¹).³³

The strong correlation observed in Figure 2b suggests that the solvatochromic shifts of the quinoline ring symmetric stretching mode can be parsimoniously described by the Stark effect using a simple Onsager solvation model. According to eqs 2 and 3, the solvatochromic response of a vibrational probe should decrease if the gas-phase dipole moment of the solute decreases. Therefore, to further test this hypothesis, we examined the solvatochromic shifts of quinoline, which has a smaller gas-phase dipole moment ($|\vec{\mu}_0| = 2.0563 \text{ D}$, as determined by DFT calculations). The solvatochromic response of quinoline's ca. 1375 cm⁻¹ ring stretching mode is only ca. 0.27 cm⁻¹/MV cm⁻¹ (Figure S4), which is roughly three times smaller than that of quinine's corresponding mode. This result is consistent with the hypothesis that the solvatochromic shifts of the quinoline ring mode of quinine can be described by the Onsager model and the Stark effect. However, it is not a definitive test since the projection of quinoline's ground-state dipole moment with respect to the 1375 cm⁻¹ mode difference dipole is not the same as that of quinine's corresponding mode.

We further tested the validity of the Onsager model to describe the vibrational solvatochromic shifts observed in the quinoline ring symmetric stretching mode by calculating the solvent reaction fields using atomistic MD simulations. Several studies^{47,61–63} have shown that classical MD force fields like Amber, OPLS/AA, and CHARMM reproduce key bulk properties of organic liquids, such as enthalpies of vaporization, surface tensions, heat capacities, and densities. Building upon these studies, Boxer and co-workers^{33,34} have employed MD simulations to calculate the electric fields felt by vibrational probes with reasonable accuracy. The simulations that we carried out utilized both the CHARMM36 and Amber ff99SB force fields. As discussed in detail in the SI, we determined the average electric fields for the same aprotic, nonaromatic solvents shown in Figure 2b. The electric fields projected onto the quinoline symmetric stretching ring mode were calculated for each snapshot using the methodology prescribed by Fried et al.³³

Overall, we find that the average electric fields calculated using Amber ff99SB and CHARMM36 qualitatively agree, although the latter shows a stronger correlation with the Onsager reaction fields (Figure S5). Table 1 compares the MD-calculated average electric fields using CHARMM36 to those determined using the Onsager model. As expected, the MD simulations indicate that polar solvents exercise a stronger average electric field on quinine's quinoline ring than less polar solvents. Despite the qualitative agreement, however, the simulations underestimate the Onsager reaction fields by a factor of ca. 2 (Figure S5). This discrepancy could derive from several factors, which we discuss in more detail in the SI.

To directly compare the MD results to the Onsager model, we uniformly scaled the CHARMM36-determined electric fields by an ad hoc scaling factor of 1.91 (Table 1 and Figure S5). Figure 2c shows the observed Raman frequencies of the quinoline ring symmetric stretching mode with respect to the scaled CHARMM36 calculated electric fields. The data recapitulate the trend observed in Figure 2b with remarkable quantitative agreement. The resultant regression line of the data shown in Figure 2c exhibits a slope (0.87 MV cm^{-1}) that essentially matches the one in Figure 2b. The excellent agreement between the data shown in Figure 2b,c suggests that the solvatochromic shifts observed for quinine's quinoline ring symmetric stretching mode in aprotic and nonaromatic solvents is sufficiently described by the Onsager model.

Effects of Hydrogen Bonding and π -Stacking on the 1370 cm^{-1} Mode.

We initially avoided using protic and aromatic solvents because they can hydrogen bond and π -stack with quinine, which can lead to electrostatic interactions not captured by the Onsager model.³² In addition, these interactions can perturb the frequencies of vibrations in a manner not described by the Stark effect. To assess the impact of π -stacking and hydrogen bonding, we examined the frequency shifts of the quinoline ring symmetric stretching mode in toluene, anisole, methanol, ethanol, and isopropanol. As shown in Figure 2b, the frequency shifts of the quinoline ring mode in these solvents deviate significantly from the linear trendline described by eq 4. For anisole and toluene, the quinoline ring mode is downshifted relative to the trendline prediction, whereas for the alcohols, the vibration is upshifted.

Our MD simulations (Figure 2c) recapitulate the systematic deviations observed in aromatic and protic solvents. The scaled MD-calculated electric field determined for anisole differs from that predicted by the Onsager model by ca. 15%, whereas fields determined for toluene and methanol differ by ca. 20%. These significant differences reinforce the inappropriateness of using the Onsager model to predict the solvent reaction fields of quinine in aromatic and protic solvent molecules. However, the most important point that our simulations reinforce is that the frequency variation of the quinoline ring symmetric stretching mode cannot be described purely in terms of a simple, linear Stark effect.

pH Dependence of 1370 cm^{-1} Mode.

Roman et al.⁵⁴ noted that protonation of the quinoline and quinuclidine moieties results in dramatic changes to quinine's Raman spectrum. As shown in Figure 3, the most dramatic spectral change occurs to the quinoline ring stretching mode, which splits into two bands located at ca. 1370 and 1390 cm^{-1} . The relative intensities of these bands are pH-dependent: as the pH increases from 2.3 to 5.1, the 1390 cm^{-1} band decreases in intensity, while the 1370 cm^{-1} band increases (Figure 3b). Because of these bands' apparent sensitivity to pH, Roman et al.⁵⁴ have suggested that the 1370 and 1390 cm^{-1} bands are diagnostic of the protonation state of the quinoline ring of quinine.

To better understand the origins of this band splitting, we performed DFT calculations and analyzed the normal mode composition of the quinoline ring symmetric stretching mode for unprotonated, monoprotonated, and diprotonated quinine (Figure 3a). Protonation of the

quinine's quinoline and quinuclidine ring moieties results in a reorganization of the eigenvector composition of the quinoline ring stretching mode (Table S2). In the case of the monoprotonated state, the normal mode composition of the vibration resembles that of unprotonated quinine, with the exception that N–H bending in the quinuclidine moiety couples significantly to motions localized in the quinoline ring. However, for the diprotonated state, the normal mode composition of the quinoline ring stretching mode changes dramatically. In contrast to the unprotonated and monoprotonated states of quinine, the most significant components of the PED for the mode in the diprotonated state are localized in only a portion of the quinoline ring (Table S2 and Figure S2).

The DFT calculations confirm that the ca. 1370 and 1390 cm^{-1} bands spectroscopically report on the protonation state of quinine's quinoline ring. Thus, the intensities of these bands can be used to determine the relative concentrations of quinine molecules with protonated and unprotonated quinoline rings. The fraction of quinine with unprotonated quinoline rings, α , can be calculated (see inset of Figure 3b) using the following equation

$$\alpha = \frac{\sigma'_{1370\text{ cm}^{-1}} I_{1370\text{ cm}^{-1}}}{\sigma'_{1370\text{ cm}^{-1}} I_{1370\text{ cm}^{-1}} + \sigma'_{1390\text{ cm}^{-1}} I_{1390\text{ cm}^{-1}}} \quad (5)$$

where $I_{1370\text{ cm}^{-1}}$ and $I_{1390\text{ cm}^{-1}}$ are the relative peak intensities of the 1370 and 1390 cm^{-1} bands, respectively. The terms $\sigma'_{1370\text{ cm}^{-1}}$ and $\sigma'_{1390\text{ cm}^{-1}}$ denote the differential Raman cross sections for the 1370 and 1390 cm^{-1} modes, respectively. The spectra shown in Figure 3 were measured in solutions containing 1% (v/v) acetonitrile. We used acetonitrile's ca. 918 cm^{-1} band, which derives from C–C stretching, as an internal intensity standard to normalize the spectra. To estimate the differential scattering cross sections of the quinine Raman 1390 cm^{-1} band, we used the following equation

$$\sigma'_i = \frac{d\sigma_i}{d\Omega} = \frac{I_i C_i}{I_r C_r} \left(\frac{d\sigma_r}{d\Omega} \right) \quad (6)$$

where $d\sigma_i/d\Omega$ is the differential cross section of the 1390 cm^{-1} quinine Raman mode and $d\sigma_r/d\Omega$ is the differential cross section of the 918 cm^{-1} C–C stretching mode of acetonitrile. C_r and C_i are the concentrations of acetonitrile and quinine, respectively. The differential cross section of the 918 cm^{-1} reference band, with 785 nm excitation, was estimated to be ca. 2.2×10^{-4} mb molecule $^{-1}$ sr $^{-1}$ by extrapolating the cross-sectional measurements of Dudik et al.⁶⁴

We use the Raman spectrum at pH 2.3, where essentially all quinine molecules are diprotonated, to estimate the differential cross section of the 1390 cm^{-1} mode. We estimate the cross section to be ca. 3.7×10^{-3} mb molecule $^{-1}$ sr $^{-1}$. The $\text{p}K_a$ values for the quinoline ring and quinuclidine head of quinine are ca. 4.3 and 8.4, respectively.⁶⁵ To estimate the differential Raman cross section of the 1370 cm^{-1} mode, we correlated the apparent normalized peak intensities at each solution pH to α . We estimate the differential cross

section of the 1370 cm^{-1} mode to be ca. $3.5 \times 10^{-3}\text{ mb molecule}^{-1}\text{ sr}^{-1}$ by extrapolating to $\alpha = 1$.

MD Simulations of Quinine–DNA Binding.

UV absorption studies suggest that quinine and its derivatives weakly bind DNA, yet a detailed molecular-level understanding of their binding remains unclear.^{10,12–18} To develop a more molecular-level picture, we conducted MD simulations to investigate the binding of quinine to DNA. Before simulations with DNA were carried out, we sought to calculate a baseline local electric field of the two quinine protonation states in an aqueous environment. Aqueous systems of mono- and diprotonated quinine were prepared by solvating quinine in boxes of water and low concentrations of acetate anions. MD simulations of these systems were performed, and the local electric fields exerted on the quinine protonation states by the solvent environment were calculated. As expected, the diprotonated state of quinine felt a stronger (unsealed) local electric field of $-48.72 \pm 18.69\text{ MV cm}^{-1}$ in aqueous solutions and the monoprotonated state had a solvent-induced field of $-39.58 \pm 17.43\text{ MV cm}^{-1}$. These values are reasonable, given that a more charged molecule will interact more strongly with a polar solvent, producing a stronger electrostatic force and thus a larger electric field.

We simulated systems composed of diprotonated quinine and a DNA double helix in aqueous solution. Two different DNA helices were used in these systems, one with 11 adenine–thymine base pairs, (AT)₁₁, and one with 11 cytosine–guanine base pairs, (CG)₁₁. Both of these DNA double helices were simulated to monitor any potential preference or selectivity of quinine in binding to DNA base pairs. The simulations were initialized with quinine 15 Å away from the center of the double helix. Our analysis of the quinine–DNA simulation trajectories reveals that diprotonated quinine favorably binds to the minor groove of the DNA double helices for both systems. The portion of the trajectories with quinine bound was extracted, and the local electric field felt by quinine in these structures was determined. Salient quinine–DNA binding interactions were abundant in the coordinate trajectory, occurring in roughly 52% of trajectory frames for the CG duplex simulation and 45% of trajectory-frames for the AT duplex simulation.

For the bound structures, we observe persistent hydrogen bonding and π -stacking interactions that are conserved throughout the relevant portions of the MD trajectories. These persistent interactions stabilize the binding of quinine to DNA. We show a representative MD snapshot of the quinine–(AT)₁₁ DNA complex in Figure 4a. In this complex, the aromatic quinoline moiety of quinine fits in the cavity between the two phosphate backbones of the DNA and A–T bases. The quinoline ring is stabilized by plane-edge ring π -stacking interactions with two bases, as well as from a persistent hydrogen bond between quinine's alcohol functional group to the N(3) unprotonated aromatic nitrogen of the adjacent adenine. In the (CG)₁₁ system, we also observed quinine interacting with the minor groove of DNA via a related but slightly different interaction. Similar to the bound structure observed for the (AT)₁₁ duplex, the quinoline ring of quinine inserts into the minor groove between the DNA backbone and sits against the two bases, forming plane-edge ring π -stacking interactions. In this complex, quinine forms two hydrogen bonds with DNA: both the alcohol and methoxy oxygens of quinine accept hydrogen bonds from the primary

amines of two guanine bases. A representative structure of this complex is shown in Figure 4b.

Interestingly, we also observe transient electrostatic interactions between the protonated amine group of the quinuclidine head of quinine and the negatively charged phosphate oxygens of DNA in both the (CG)₁₁ and (AT)₁₁ systems (Figure S6). These interactions appear to stabilize the DNA-bound quinine geometry by anchoring the otherwise freely rotating quinuclidine head group. When the quinuclidine head electrostatically binds to the phosphate backbone in the minor groove, we hypothesize that quinine retains conformational flexibility to dynamically search for more rigid and stable DNA-bound structures.

We calculated the local electric field felt by quinine in the minor groove bound structures of (AT)₁₁ and (CG)₁₁. For these calculations, solvent and DNA atoms were used to model the local environment around quinine's quinoline ring moiety. In the quinine-(AT)₁₁ complex, we calculated the local electric field to be $-64.8 \pm 19.11 \text{ MV cm}^{-1}$, ca. 16 MV cm^{-1} higher than that in aqueous solution. For quinine bound to (CG)₁₁, an effective electric field felt by the quinoline ring moiety is $-61.65 \pm 19.37 \text{ MV cm}^{-1}$, ca. 13 MV cm^{-1} higher than that for aqueous quinine.

These results indicate that diprotonated quinine binds favorably in the minor groove of the (AT)₁₁ and (CG)₁₁ helices. To further investigate this result, we performed umbrella-sampling simulations on the two different quinine-DNA systems to calculate a free energy of binding (G_{binding}). We accomplished this by translating the quinine out of the binding site in the minor groove of DNA at discrete distances until it was fully solvated. Using umbrella-sampling free-energy calculations for the binding pathway, we determined that the G_{binding} for the quinine-(AT)₁₁ complex is ca. $-11.9 \text{ kcal mol}^{-1}$ and ca. $-11.7 \text{ kcal mol}^{-1}$ for the quinine-(GC)₁₁ complex. These free-energy calculations confirm that diprotonated quinine intercalates favorably to the minor groove of DNA through the formation of t-shaped, plane-edge ring stacking interactions, as well as preferential hydrogen bonds to adenine and guanine.

Raman Spectra of Quinine-DNA Complexes.

We utilized the quinoline ring symmetric stretching mode as a probe to experimentally interrogate the binding and possible intercalation of quinine to DNA. Figure 5 shows the Raman spectrum of quinine in the presence of calf-thymus DNA (Figure 5a), as well the spectra of unbound quinine (Figure 5b) and DNA (Figure 5c). We measured the spectra under acidic conditions (pH 4.3), in sodium acetate buffer, to maintain the solubility of quinine. Upon mixing the quinine and DNA solutions, an increase in viscosity was observed (a more detailed discussion on the rheology of these solutions is available in the SI, Figure S7). This is in congruence with Estensen et al.⁶⁶ and Jones et al.'s¹⁷ observations, who report increases in viscosity upon mixing quinine and chloroquine solutions with DNA. This change in the viscoelastic profile of the DNA solution upon introduction of quinine suggests an interaction between quinine and DNA.⁶⁶

The spectra of quinine and quinine–DNA are dominated by the ca. 1370 and 1390 cm^{-1} bands of the quinoline ring stretching modes. In contrast, the spectrum of DNA shows only a weak, asymmetric band at ca. 1375 cm^{-1} , which derives from the ring stretching modes of the thymine, guanine, and adenine bases.⁶⁷ The main spectral change that occurs in DNA-bound quinine is an apparent increase in the 1390 cm^{-1} band relative to the 1370 cm^{-1} band. To better highlight the changes, we subtracted the spectra shown in Figure 5b,c from the quinine–DNA spectrum depicted in Figure 5a. The resulting difference spectrum (Figure 5d) shows a negative feature at 1370 cm^{-1} and a positive feature at ca. 1380 cm^{-1} . The presence of these features demonstrate that the quinine–DNA spectrum (Figure 5a) cannot be modeled simply as a linear combination of the monomeric quinine and DNA spectra (Figure 5b,c).

The difference spectrum indicates that, in the presence of DNA, there is a decrease in the concentration of monoprotonated quinine and an increase in the concentration of diprotonated quinine. On the basis of the intensity changes, we estimate that the concentration of quinine with protonated quinoline rings increases roughly by 11%. Interestingly, the positive feature in the difference spectrum is downshifted ca. 10 cm^{-1} from the characteristic 1390 cm^{-1} mode of monomeric diprotonated quinine, which signals π -stacking interactions. Therefore, we interpret the Raman difference spectrum as follows: quinine binds to DNA and the quinoline ring amine group becomes concomitantly protonated. The protonated quinoline ring forms π -stacking interactions with the DNA base pairs. On the basis of the magnitude of the intensity changes in the difference spectrum, we roughly estimate that one quinine molecule intercalates per every 25 base pairs. This binding density corresponds to an apparent association constant (K_a) of $5.2 \times 10^5 \text{ M}^{-1}$.

A competing interpretation of the downshifted ca. 1380 cm^{-1} feature in Figure 5d is that it derives from stable populations of π -stacking quinine molecules that occur in the presence of DNA. Such a situation can occur, for example, if two neighboring quinine molecules electrostatically bind the phosphate backbone of DNA via their protonated quinuclidine head groups and form π -stacking interactions between their quinoline rings. To test this hypothesis, we sought to determine the spectral signatures of quinine molecules forming complexes with each other through π -stacking interactions. We examined these signatures by measuring the Raman spectra of quinine at different concentrations ranging above, below, and at its saturation point (Figure S8). At low concentrations (6.15 mg mL^{-1}), we expect quinine to be monomeric, whereas saturated and supersaturated solutions (12.3 and 19.4 mg mL^{-1} , respectively) are expected to contain stable populations of interacting quinine molecules. We find that the Raman spectrum of a saturated solution of quinine (Figure S8b) is essentially identical to that of monomeric quinine (Figure S8a). The only difference is that the spectrum of the saturated solution shows an increase in the 1370 cm^{-1} band relative to the 1390 cm^{-1} band, which is due to self-buffering of the quinine. In supersaturated solutions, the 1390 cm^{-1} band downshifts by ca. 3 cm^{-1} (Figure S8c) due to quinine molecules forming π -stacking interactions. In contrast, a dispersion of quinine composed of high-molecular-weight aggregates exhibits a Raman spectrum (Figure S8d) that shows no 1390 cm^{-1} mode and a 1370 cm^{-1} mode that is downshifted by ca. 5 cm^{-1} due to π -stacking interactions that occur between monoprotonated quinine molecules. The magnitudes of the 3–5 cm^{-1} downshifts observed for mono- and diprotonated quinine molecules in Figure

S8c,d are too small to assign the ca. 1380 cm⁻¹ feature observed in Figure 5d to inter-quinoline ring π -stacking interactions. On the basis of these observations, we can rule out formation of stable quinine–quinine π -stacking due to the presence of DNA.

We also ruled out the possibility that the 1380 cm⁻¹ feature in Figure 5d derives from electrostatic interactions between the cationic quinoline ring of diprotonated quinine molecules with the phosphate backbone of DNA. To investigate this possibility, we measured the Raman spectrum of quinine in a high-ionic-strength (1 M) solution of NaH₂PO₄ (Figure S9). In this solution, the concentration of NaH₂PO₄ is ca. 1000× higher than that of quinine. However, the 1370 and 1390 cm⁻¹ modes of quinine surprisingly do not exhibit any significant frequency shifts, indicating that the quinoline ring moieties of mono- and diprotonated quinine do not form stable salt bridge interactions with phosphate ions. The only significant spectral change is that the relative intensity of the 1390 cm⁻¹ band increases compared with the 1370 cm⁻¹ band due to the change in the solution pH upon addition of NaH₂PO₄.

We further attempted to validate our interpretation of the Raman difference spectrum by monitoring quinine–DNA binding using fluorescence spectroscopy (see the SI for details). The fluorescence of quinine is expected to quench in the presence of DNA due to its intercalation into the base pairs.¹⁹ At low concentrations (1.5×10^{-3} to 1.5×10^{-2} mg mL⁻¹) of quinine and DNA (Figure S10c,d), we see a fluorescence reduction by ca. 5–8%, qualitatively consistent with the Raman data. However, at higher concentrations (0.15–1.5 mg mL⁻¹), we observe a significantly larger decrease in the fluorescence signal (Figure S10a,b). In particular, we observe a ca. 40% apparent decrease in the fluorescence at the same concentrations of DNA–quinine used in the Raman measurements.

One compelling reason for the apparent discrepancy between the Raman and fluorescence measurements could stem from dynamic self-quenching that occurs between transiently interacting quinine molecules at high concentrations. Such self-quenching phenomena has been reported by Krimer et al.⁶⁸ for quinine solutions prepared at concentrations similar to that used in our Raman and fluorescence measurements. In addition, Edward et al.⁶⁹ observed selfquenching for small quinine-functionalized polycarbonate polymers, where π -stacking interactions between the quinoline rings of neighboring covalently linked quinine molecules can occur. We envision that dynamic self-quenching occurs if the local concentration of cationic mono- and diprotonated quinine molecules increases in the vicinity of DNA. This can occur if quinine molecules electrostatically bind the negatively charged phosphate backbone of DNA. On the basis of our Raman data (Figures 5 and S7), these electrostatic interactions do not occur between the DNA phosphate backbone and quinine's quinoline ring. A more likely scenario is that the quinuclidine head group of quinine weakly binds the phosphate backbone through transient electrostatic interactions, as observed in our MD simulations (Figure S6).

Wesucha-Birczyńska and Nakamoto^{27,2} propose that electrostatic interactions between the quinuclidine head group and DNA occurs for the quinine derivative, cinchonine. Their supporting evidence, however, is indirect since they only observe a small Raman frequency shift in the symmetric stretching mode of the DNA phosphate backbone and not in any

cinchonine bands. For quinine, we also see no clear spectroscopic evidence supporting this hypothesis, although we do not have a good vibrational probe to specifically monitor the local environment around the quinuclidine moiety. On the basis of the lack of clear spectroscopic evidence, the electrostatic interactions between the quinuclidine head group and DNA must be very weak and transient if they indeed occur.

Estensen et al.⁶⁶ used UV absorption and viscometric measurements to investigate quinine–DNA binding. Their data indicates that quinine binds DNA in two different regimes. One binding regime occurs at low (sub-millimolar) quinine concentrations, whereas the other occurs at high concentrations (millimolar). At low concentrations, they observe that one molecule of quinine binds per 40 base pairs. They hypothesize that this binding mode corresponds to quinine intercalating into DNA through π -stacking interactions. This binding density is similar to but slightly lower than that estimated from our Raman measurements. The discrepancy between the two values could stem from the fact that our measurements are performed under acidic conditions, whereas Estensen et al.'s⁶⁶ measurements are performed at pH 7.4. The second binding mode that Estensen et al.⁶⁶ observe occurs at millimolar concentrations, where one quinine molecule binds approximately every 8–9 base pairs. Estensen et al.⁶⁶ speculate that this second binding regime corresponds to quinine molecules electrostatically interacting with the DNA phosphate backbone via their quinuclidine moieties, presumably in the minor groove. They argue that this binding mode can account for the observed viscosity change at millimolar concentrations that occurs when quinine and DNA are mixed together in solution.

Overall, it seems reasonable that cationic quinine molecules form transient and weak interactions with the phosphate backbone of DNA, which would give rise to dynamic selfquenching of the fluorescence. However, it is ultimately difficult to definitively conclude on what causes the 40% reduction in fluorescence at high concentrations of quinine and DNA. This is because electronic spectroscopies such as UV absorption and fluorescence lack the chemical specificity of vibrationally resolved methods such as Raman spectroscopy. This makes it difficult to clearly discern the molecular-level origins of the observed absorption and fluorescence intensity changes without extensive a priori knowledge. This is especially the case for quinine, whose fluorescence intensity changes are confounded by several factors, including concentration-dependent self-quenching, pH, chloride ions, DNA, and other environmental factors. In contrast, the Raman measurements are not confounded by these competing factors and clearly indicate that quinine molecules intercalate into the DNA by forming π -stacking interactions between its protonated quinoline ring and the DNA base pairs.

CONCLUSIONS

We show that quinine's prominent quinoline ring symmetric stretching mode is an excellent spectroscopic probe for monitoring the local chemical environment. In solvents that do not interact strongly with quinine, the solvatochromic shifts of this mode can be quantitatively understood in terms of the Stark effect through the use of the Onsager model, which simplistically treats the solvent environment as a continuum dielectric. In contrast, the Onsager model and Stark effect fail to adequately describe the frequency shifts observed in

the quinoline ring symmetric stretching mode for solvating molecules that can interact with quinine through hydrogen bonding and π -stacking interactions. Despite this, our solvatochromic shift measurements establish a qualitative benchmark for interpreting spectroscopic data to assess the interaction of quinine's quinoline ring moiety with its environment through electrostatics, hydrogen bonding, or π -stacking.

Using the environmental sensitivity of this vibrational probe, we investigate the binding of quinine to calf-thymus DNA. The Raman data indicates that the intercalation of quinine into the DNA base pairs is accompanied by protonation of the tertiary amine of the quinoline ring. The predominant binding mode of quinine to DNA is through π -stacking interactions between the quinoline ring and the base pairs, rather than electrostatic interactions with the phosphate backbone. On the basis of the Raman spectral changes, we estimate that, under acidic conditions, roughly one quinine molecule intercalates per 25 base pairs of DNA. The conclusions that we draw from our Raman measurements are qualitatively reinforced by MD simulations. However, it is important to note that the MD simulations that we conducted are far from exhaustive in terms of probing quinine–DNA complexation. It is possible that the quinine–DNA binding events observed in the simulations are precursors to stronger intercalation events. In addition, the electrostatic interactions observed in the simulations between the quinuclidine head of quinine and the phosphate backbone may function as an initiator of quinine intercalation into DNA. Future work should investigate these interactions further and determine any potential functional significance.

We expect that the work presented here will enable future studies investigating quinine's biological mechanism of action against malarial parasites. The chemical specificity of vibrationally resolved methods such as Raman spectroscopy offers tremendous advantages over traditional fluorescence and absorption-based methods in probing the molecular interactions of quinine with its environment. In particular, the quinoline ring symmetric stretching mode can serve as a spectroscopic probe to interrogate how quinine disrupts hemozoin biocrystallization or alkalinizes acidic food vacuoles and lysosomes in *Plasmodium* spp. parasites. Our work presented here can be easily extended to other Cinchona alkaloids and compounds such as chloroquine and mefloquine, which show correspondingly strong Raman bands near ca. 1370 cm^{-1} that derive from similar quinoline ring stretching modes. Such a comparative study could elucidate why chloroquine and mefloquine are more potent antimalarials than quinine or why some *Plasmodium* strains develop drug resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Raman spectra of (a) quinine and (b) quinoline in methanol excited at 785 nm and atomic displacements of selected quinine vibrational modes for the ca. (c) 1640 cm⁻¹, (d) 1630 cm⁻¹, (e) 1580 cm⁻¹, (f) 1370 cm⁻¹, (g) 820 cm⁻¹, and (h) 770 cm⁻¹ Raman bands. The spectra were baseline-corrected to remove the broad fluorescence background, and the spectral contributions of methanol were subtracted. The spectra are normalized with respect to their maximum peak intensities. The * symbol shows an artifact of subtracting the spectral contribution of methanol.

Figure 2.

Solvatochromic data for quinine's ca. 1370 cm⁻¹ quinoline ring stretching mode. (a) Raman spectra of quinine's 1370 cm⁻¹ mode in six different aprotic solvents (from left to right: acetonitrile, dimethyl sulfoxide, acetone, dichloromethane, tetrahydrofuran, and chloroform). The spectra are normalized with respect to their peak maxima. (b) Plot of the 1370 cm⁻¹ quinoline ring stretching mode frequency as a function of the Onsager reaction field for different aprotic, protic, and aromatic solvents. Standard error bars for the frequencies are shown for each point and are 0.1 cm⁻¹. (c) Plot of ca. 1370 cm⁻¹ mode frequency as a function of the average MD-calculated electric fields projected onto the vibration. The MD-calculated electric fields (CHARMM36) were scaled by a factor of 1.91 to enable a direct comparison with the Onsager reaction fields shown in (b). The error bars for the electric fields were obtained by computing the standard deviation of the local electric field calculated from the MD simulations. The numbered points in (b) and (c) denote the following solvents: (1) gas phase, (2) chloroform, (3) tetrahydrofuran, (4) dichloromethane, (5) acetone, (6) acetonitrile, (7) dimethyl sulfoxide, (8) toluene, (9) anisole, (10) isopropanol, (11) ethanol, and (12) methanol. The gas-phase frequency of the ca. 1370 cm⁻¹ mode was obtained from Sen et al.⁷⁰

Figure 3.

Sensitivity of the quinoline ring stretching mode to pH for dilute quinine in aqueous solution. (a) chemical structures of unprotonated, monoprotected, and diprotected quinine. (b) Raman spectra were measured at pH 2.3, 3.3, 4.4, and 5.1. The arrows point toward increasing pH. Inset: Correlation between α calculated using the pK_a of quinine's quinoline ring amine group (α_{calc}) and Raman measurements (α_{Raman}). The spectra were normalized with respect to the intensity of the ca. 918 cm⁻¹ band of acetonitrile, which we used as an internal intensity standard.

Figure 4. Representative snapshots from the MD trajectories of quinine binding to (a) (AT)₁₁ and (b) (GC)₁₁ DNA helices.

Figure 5.

Raman spectra of (a) quinine and DNA mixed together in solution, as well as free (b) quinine and (c) DNA. The difference spectrum shown in (d) was calculated by subtracting the spectra in (b) and (c) from the spectrum shown in (a). All samples were prepared in sodium acetate buffer at pH 4.3. The spectra were all normalized to the ca. 900 cm^{-1} C–C stretching mode of acetate. The spectra shown here were conservatively smoothed for clarity using a Savitzky–Golay filter⁷¹ with a fourth-order polynomial over an 11-point range.

Table 1.

Comparison of Onsager Solvation and CHARMM36 MD-Calculated E-fields

solvent	ϵ	Onsager model (MV cm ⁻¹)	raw MD E-field (MV cm ⁻¹)	scaled MD E-field (MV cm ⁻¹)	% difference ^a
chloroform	4.81	-8.6	-6.0	-8.9	3.9
tetrahydrofuran	7.58	-10.2	-6.5	-9.7	5.0
dichloromethane	8.93	-10.7	-7.1	-10.6	0.6
acetone	20.7	-12.4	-8.2	-12.2	1.3
acetonitrile	37.5	-13.0	-8.5	-12.7	2.5
dimethyl sulfoxide	46.7	-13.1	-9.2	-13.8	4.8

^aDifference = $|E_{\text{Onsager}} - E_{\text{Scaled MD}}|/E_{\text{Onsager}} \times 100\%$.