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Electronic Structure and Partial Charge Distribution of Doxorubicin in Different Molecular Environments

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The electronic structure and partial charge of doxorubicin (DOX) in three different molecular environments—isolated, solvated, and intercalated in a DNA complex—are studied by first-principles density functional methods. It is shown that the addition of solvating water molecules to DOX, together with the proximity to and interaction with DNA, has a significant impact on the electronic structure as well as on the partial charge distribution. Significant improvement in estimating the DOX–DNA interaction energy is achieved. The results are further elucidated by resolving the total density of states and surface charge density into different functional groups. It is concluded that the presence of the solvent and the details of the interaction geometry matter greatly in determining the stability of DOX complexation. Ab initio calculations on realistic models are an important step toward a more accurate description of the long-range interactions in biomolecular systems.

1. Introduction

Doxorubicin (trade name Adriamycin, abbreviated DOX) is a well-known anthracyclic chemotherapeutic used to treat a variety of cancers including acute leukemia, lymphoma, multiple myeloma, and a range of stomach, lung, bladder, bone, breast, and ovarian cancers.[1] DOX is a potent cytotoxic agent that limits the growth of cancer cells by induction of apoptosis.[2] Biochemical evidence suggests that it primarily works by blocking replication and transcription through complex formation with DNA and interfering with the enzyme topoisomerase II.[3] Several attempts have been made to understand the key features responsible for the specific biological activity of this compound, particularly its interaction with DNA.[4] The purpose of the present work was to study and understand the partial-charge distribution and electronic structure of DOX in different molecular environments, with the goal to provide a framework for understanding long-range interactions involving DOX or other DNA-intercalating biomolecules. Although this work focuses on DOX–DNA interactions, the knowledge gained can be translated to biomolecular interactions more generally.

Whereas knowledge of the electronic structure and charge distribution of biomolecules is important in explaining bioactivity,[5] quantitative information is in general seldom available. This situation has started to change in recent years, due to the more rigorous computational studies that have emerged and continue to expand.[6] Understanding electronic properties of complicated biological macromolecules gives insight into the interactions between them. These are essential for unraveling important life processes such as DNA replication, transcription, and repair. It also enables tools to be developed for their control and modification through rational design of drugs and other mesoscale structures that improve the functionalities that depend on them.[7] Advanced quantum mechanical ab initio methods are essential for accurate calculation of the electronic structure of any molecule.[8] However, most ab initio calculations of biomolecular systems focus on small fragments of molecular structure or are limited to well-known structural subunits, and they seldom venture into the realm of more realistic biomolecules that require robust large-scale computations.[9] In addition, the most interesting and relevant biomolecular systems are always bathed in complex aqueous environments,

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which further fundamentally exacerbate the complexity of computational studies.[10]

To advance our knowledge of complicated biomolecules, we carried out ab initio calculations of the electronic structure and partial-charge distribution of DOX in three different molecular environments, which can be considered to be different solution conditions, to better understand its long range interactions with other moieties and its bioactivity. Whereas the electronic structure and optical properties of biomolecules are important for elucidating the long-range van der Waals/London dispersion interactions between them,[11] their partial-charge distribution is of paramount importance because of its imprint on the electrostatic and polar components of the long-range interactions.[12] It is the latter that guide the molecules into their docking configuration and ensure the stability of the molecular complex, which also depends on its detailed solution environment.[13] To properly capture the role of different molecular environments in the interaction between DOX and other biomolecules (e.g. DNA), we explicitly studied the following modifications of DOX: 1) DOX in vacuum (also referred to as isolated DOX), 2) solvated DOX in water boxes, and 3) DOX–DNA complex in the molecular environment, based on its crystal structure.

Solvent molecules (water) play a crucial role in governing the structure, stability, dynamics, and function of biomolecules. They are primarily responsible for hydrophobic and/or hydrophilic solvent-mediated interactions[14] through the formation of a network of hydrogen bonds.[15] Thus, investigation of the long-range electrostatic and van der Waals/London dispersion interactions must include the most important features of the molecule–solvent interactions to an extent that is still computationally tractable.[16,17] This solvent effect was investigated fully by comparing the electronic properties of an isolated DOX molecule with those of a solvated DOX molecule embedded in a water box.

Methods

Structural Models

We report the results of the electronic structure and the partial-charge distributions of DOX in the above-stated three different molecular environments. We started with the isolated DOX molecule (model 1). The molecular geometry of DOX ($C_{27}H_{29}NO_{11}$, 68 atoms) was obtained from PUBCHEM (CID: 31703).[18] It consists of tetracyclic quinoid aglycone adriamycione (planar chromophore) linked with the amino sugar daunosamine. The planar chromophore has three aromatic rings created by a series of alternating single and double bonds. The amino sugar is a sugar molecule in which the hydroxyl group is replaced by an amino group. Figure 1a depicts the structure of DOX in ball-and-stick and Lewis forms.

In model 2, the isolated DOX molecule is positioned in a rectangular cell of dimensions 28.60×23.65×18.26 Å containing 255 water molecules, as described by the TIP3P[19] water model implemented in the Chimera software.[20] The TIP3P water model is a simple three-site model with three interaction points corresponding to the three atoms of the water molecule. Each site has a point charge, and the site corresponding to the oxygen atom has assigned Lennard–Jones parameters. The O–H bond length and H-O-H bond angle are set to 0.95 Å and 104.52°, respectively. The water molecules were added around the DOX molecule by using AmberTools[21] incorporated in the Chimera software. There are a total of 833 atoms in this model of solvated DOX, which we designate as model 2b. To investigate possible variations of modeling results with different configurations of water as medium, we constructed two additional models of DOX in a water box with different sizes, one of which was smaller and the other larger than model 2b. They are labeled model 2a and model 2c, respectively. Model 2a has dimensions of 26.00×22.00×16.00 Å and contains 196 water molecules, and models 2c has dimensions of 29.00×24.50×18.50 Å with 300 water molecules. The total number of atoms including the DOX molecule in models 2a, 2b, and 2c are 656, 833, and 968, respectively. All three models were fully relaxed by using VASP (see below).

Next, the structure of the DOX–DNA complex (model 3) was taken from the Protein Data Bank (PDB ID: 1D12).[22] This is an experimental structure obtained by X-ray diffraction with a resolution of 1.70 Å at 288 K.[23] The structure of the DOX–DNA complex consists of two DOX molecules, a segment of DNA [$(CGATCG)_2$], 112 water molecules, two spermine ($C_{10}H_{26}N_4$) molecules and two Na atoms. The tetragonal crystal structure in space group $P4_12_12$ (no. 92) contains a total of 932 atoms. To ensure that the PDB structure was of sufficient accuracy for ab initio calculations, we again relaxed the DOX–DNA structure using VASP (see below). Figure 1a–c show schematic representations of these three models of DOX used in the calculation: isolated DOX, solvated DOX in a water box, and the fully relaxed DOX–DNA complex, respectively. Figure 1d shows the structure of DNA plus spermine without DOX and water molecules for better visual clarity of Figure 1c.

Methods of Calculation

Ab initio calculations of the electronic structure of DOX in different molecular environments employed the Vienna ab initio simulation package (VASP) for structural relaxation. VASP is based on density functional theory (DFT)[24,25] and has been highly successful for geometric optimization. In the present study, we used the projector augmented wave method with the Perdew–Burke–Ernzerhof potential[26] for the exchange correlation functional within the generalized gradient approximation. For electronic relaxation, a relatively high energy cutoff of 500 eV was adopted with the electronic convergence criterion set at 10^{-5} eV. For ionic relaxation, we set the force-convergence criterion to be 10^{-3} eV Å^{-1}. Since a large periodic supercell was used in the calculation, we used one *k* point at the zone center for a single-point calculation, which is more than sufficient for a large biomolecule. All VASP calculations were carried out at the National Energy Research Scientific Computing (NERSC) facility.

The orthogonalized linear combination of atomic orbitals (OLCAO) method was used to calculate electronic structures and partial-charge distributions of the various DOX models. The OLCAO method is an all-electron method based on the local density approximation[27,28] of DFT. It uses the atomic orbitals expanded in Gaussian-type orbitals (GTO) in the basis expansion. This method is particularly efficient for calculating the electronic structure of large complex systems, especially in the case of biomolecules.[29] The OLCAO method has been employed in the study of many other complex systems, such as inorganics,[30] organics,[31] supercooled water,[32] and biomaterials,[33,34] in the last decade. In the present calculation, a full basis, which consisted of the core orbitals, occu-

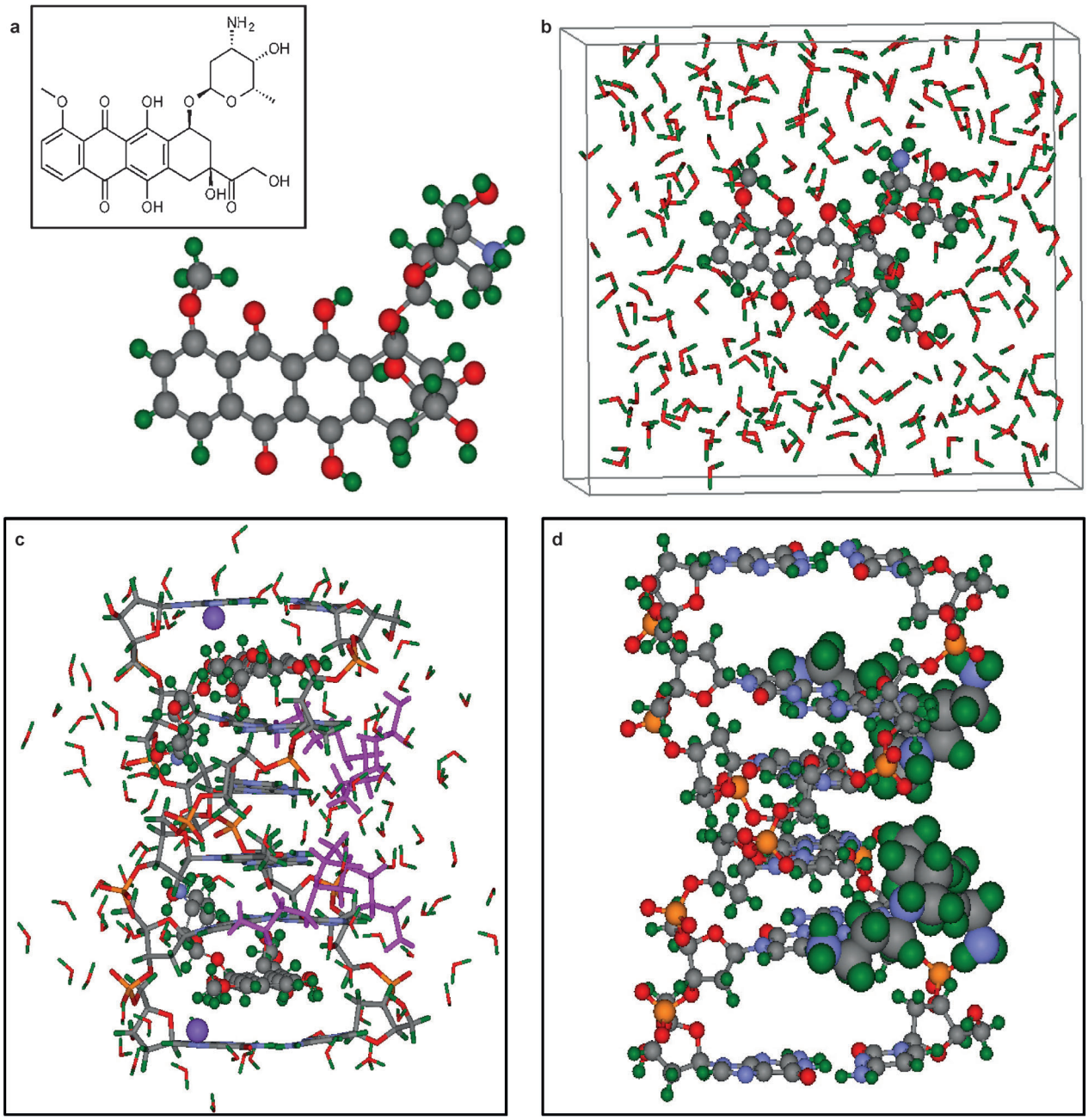

Figure 1. Structural modes of a) Isolated DOX (inset: the Lewis structure of DOX), b) DOX in a water box, c) DOX–DNA complex, and d) DNA and spermine in (c). O red, C gray, N blue, H green, Na violet, P orange. The water molecules are shown as sticks.

pied valence orbitals, and the next empty shell of unoccupied orbitals for each atom, was used for the determination of the self-consistent potential and calculations of the density of states (DOS). A minimal basis was used for the separate calculation of partial charges.

For the electronic structure calculation of the DOX models, we focused on the electronic DOS and partial-charge distribution on DOX. The total DOS (TDOS) was obtained from the energy eigenvalues after solving the final Kohn–Sham equation. We further resolved the TDOS into partial DOS (PDOS) for different groups of atoms, which can facilitate the interpretation of the electronic-structure results.[27,28] The gap between the HOMO and the LUMO is an important physical quantity for the electronic structure. The HOMO–LUMO gap can be easily identified from the TDOS and PDOS plots. The partial charge on the atom is defined as the charge deviation ΔQ of the neutral atom (Q_0) from the calculated effective charge Q^* in units of electron charge, or $\Delta Q = Q_0 - Q^*$ (i.e. $-$ve ΔQ = gain of electrons or electronegative and $+$ve ΔQ = loss of electron or electropositive). The quantitative information on partial charge is important in estimating the electrostatic component of the total intermolecular interaction potential and the effect of the presence of solvents. Q^* is calculated according to the Mullikan population analysis[35] by means of Equation (1):[36]

$$Q_\alpha^* = \sum_{i,\alpha} \sum_{n\,occ} \sum_{j,\beta} C_{i\alpha}^{*n} C_{j\beta}^{n} S_{i\alpha,j\beta} \qquad (1)$$

where $C^n_{j\beta}$ are the eigenvector coefficients of the nth state, jth orbital, and βth atom, and $S_{i\alpha,j\beta}$ are the overlap integrals between the ith orbital of the αth atom and jth orbital of the βth atom. We calculated the atomic partial charges on every atom in the three models. The partial charge on each structural group can be obtained by adding the ΔQ values of all atoms in that group.

Knowledge of accurate partial-charge distributions in molecules is essential for determining the electrostatic energies (including hydrogen bonding) in molecular simulations, which is an important tool in computational biophysics. Currently, these values are usually obtained by empirical or semi-empirical means, and this introduces a large degree of uncertainty for a specific complex biomolecular system such as DOX–DNA. An additional serious drawback in the current approaches used in molecular simulations with fixed partial charges is that it is quite difficult to readjust in response to the change in electrostatic environment, such as the presence of solvents. Since the charges are not allowed to readjust to the environment, another remedial strategy is required, such as incorporating a dielectric constant of the medium in the interaction potential, which leads to additional uncertainties in the calculations. To overcome this quandary, we propose using the more accurate partial charges calculated by the ab initio quantum mechanical method and used in the prevailing MD packages as a first step towards a more accurate and efficient way of describing the dynamic effects and long-range interactions in complex biomolecular systems.

The effect of using ab initio results of the electronic structure of DOX in three different molecular environments can be assessed by using the NAnoscale Molecular Dynamics (NAMD) code through the Visual Molecular Dynamics (VMD) graphics program[37] as a post-OLCAO calculation that uses the calculated partial charges. This enables us to estimate the energy of binding of DOX to DNA in model 3. NAMD implies a CHARMM force-field parameter. The CHARMM force field is divided into a topology file, which is needed to generate the protein structure file, and a parameter file, which supplies specific numerical values for the generic CHARMM potential function. The topology file defines the atom types used in the force field, as well as the atom names, types, bonds, and partial charges of each type of residue. The parameter file provides a mapping between bonded and nonbonded interactions involving the various combinations of atom types found in the topology file and specific spring constants and similar parameters for all of the bond, angle, dihedral, improper, and van der Waals terms in the CHARMM potential function. A parameter file was built with the appropriate energy, length, and angle values specified for the bonding between the atoms of doxorubicin based on data tables available in the Chem3D software package.[38] The associated topology file was edited to include the DOX atoms and to modify the values for DNA by using the partial-charge distribution determined above. The energy between DOX and DNA was then determined by using the NAMD Energy simulation plugin.

2. Results and Discussion

2.1. Doxorubicin in Vacuum

The calculated TDOS for the isolated DOX molecule in the energy range −25 to 25 eV is shown in Figure 2a, which shows a HOMO–LUMO gap of 3.24 eV with a defectlike gap state at 2.18 eV. The TDOS spectrum is the broadened version of the histogram plot of the energy eigenvalues and appears somewhat spiky due to the relatively small number of atoms

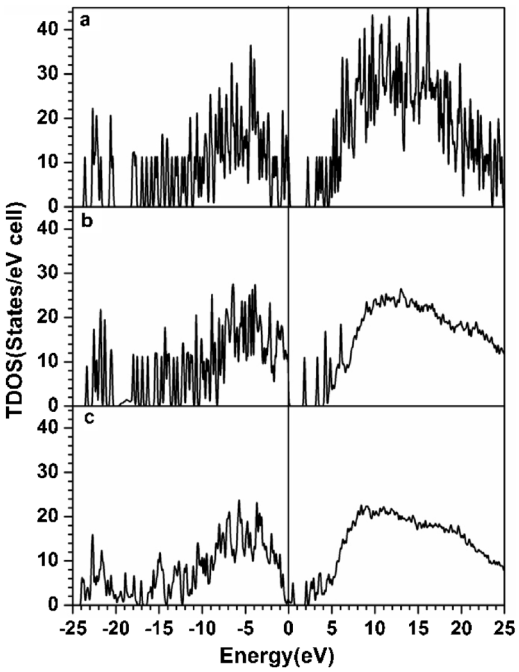

Figure 2. Calculated TDOS for DOX in different environments. a) Isolated DOX. b) DOX in a water box. c) DOX–DNA complex.

of the DOX molecule. The calculated atomic partial charges on every atom in DOX are shown in Figure 3a with numerical values for each of 68 the atoms in the molecule (left column) and their color representation (right column). In DOX, all O and N atoms are electronegative and all H atoms are electropositive. The C atoms can be electropositive or electronegative depending on their local bonding characteristics in the structure. The C atoms that are only bonded with O and C atoms are always electropositive, those that are only bonded with C atoms are less electropositive with values close to zero, and those that are bonded with other C and H atoms are always electronegative, whereas those that are bonded with H, O, and C atoms are less electronegative. DOX has only one N atom, which is the most electronegative atom with a partial charge of −0.82 e. The distribution of partial charges of O and H atoms are less variable than those of C atoms. Thus, the distribution of the partial charges on individual atoms reveals a lot about their local bonding environment. As is standard for any ab initio calculation on a neutral system, the total partial charge on the isolated DOX molecule is zero.

2.2. Solvated Doxorubicin

To study solvation effects, we placed the DOX molecule in a rectangular water box using Chimera software, and then relaxed the structure with VASP. We built three models with different sizes, numbers, and orientations of water molecules and labeled them as models 2a, 2b, and 2c in increasing order of size. The calculated electronic structures and atomic partial-charge distributions show minor variations reflecting the statistical nature of the solvated models for molecules in an aqueous environment with associated fluctuations. Ab initio calcula-

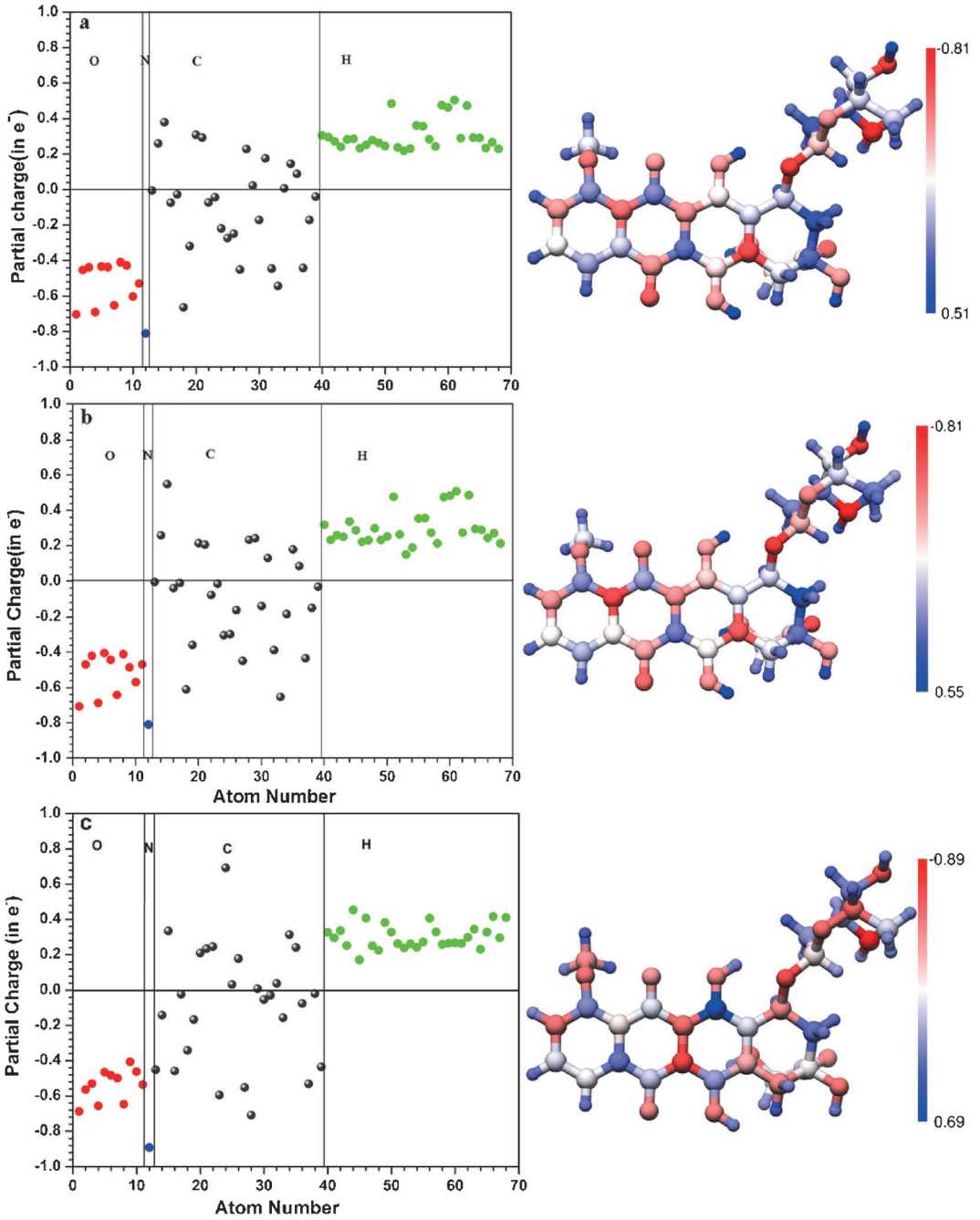

Figure 3. Calculated atomic partial-charge distribution on DOX for a) pure DOX, b) DOX in a water box, and c) DOX–DNA complex. Left column: numerical values for each atom. Right column: Color representation of the atomic partial charge.

tions on a large number of solvated models are far beyond the scope of current work. Table 1 summarizes charge distributions on three solvated models. We found that the total partial charge on DOX in solvated models 2a, 2b, and 2c are 0.077 e, 0.123 e, and 0.145 e respectively. Therefore, we chose model 2b as a reasonable representation of solvation effects for further discussion.

The calculated TDOS of solvated DOX (model 2b) without water is shown in Figure 2b. The calculated TDOS of model 2b with water is shown in Figure 4 and is resolved into separate PDOS for water and the DOX molecule. The contribution from

Table 1. Sums of the atomic partial charges on DOX in different molecular environment.

Model	Isolated DOX	DOX in water box			DOX in DNA complex
		2a	2b	2c	
$\Sigma(\Delta Q)$ [e]	0.000	0.077	0.123	0.145	−0.176
no. of atoms	68	656	833	968	932
no. of water molecules	0	196	255	300	112

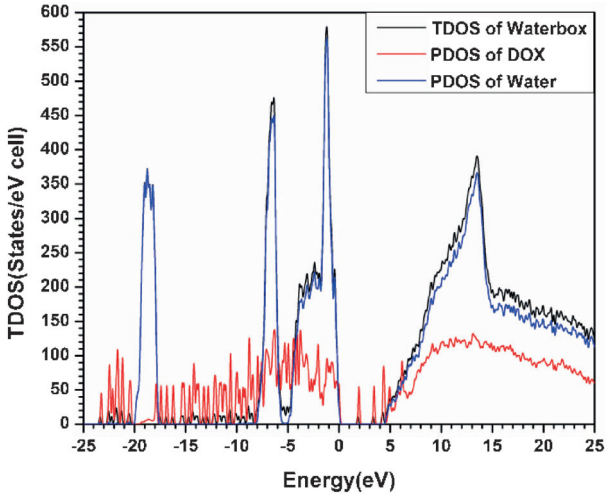

Figure 4. Calculated TDOS and PDOS of DOX in a water box. Note that the DOS of DOX is only a small fraction of the TDOS because of the presence of a large amount of water molecules. The PDOS of DOX is multiplied by a factor of five for visual clarity.

DOX in the water box is much smaller than that of the solvent, since the model contains 255 water molecules. (The PDOS for DOX in Figure 4 is multiplied by a factor of 5 to increase visual clarity.) The calculated HOMO–LUMO gap for solvated DOX model 2b is 4.20 eV with two defectlike states at 1.94 and 3.37 eV. The PDOS of water in Figure 4 shows three sharp peaks at −1.1, −6.3, −18.5 eV, a shoulder around −3.0 eV in the occupied valence-band region, and a sharp peak at 13.5 eV in the unoccupied conduction-band region.

We also calculated the atomic partial charges on solvated DOX (model 2b). The partial charge ΔQ for N changed from −0.82 e (model 1) to −0.81 e (model 2b). Similarly, the changes in the charges on the O and H atoms of DOX in the solvated model (model 2b) are also very small. On the other hand, the distributions of ΔQ for C atoms are somewhat different, that is, the solvent molecules in model 2b appear mostly to affect the C atoms that engage in different local interactions with vicinal water molecules. On the whole, the charge state of the DOX molecule in a water box (model 2b) changes from neutral in the case of isolated DOX (model 1), to an electropositive value of 0.123 e, which indicates that electron charge has been slightly transferred from the DOX molecule to the water medium due to the weak interactions. Figure 3b shows that the atomic partial charge on each atom in the DOX molecule of solvated model 2b is only slightly changed from that of the isolated DOX molecule (model 1) with a few exceptions for the C atoms, but the overall qualitative feature of the partial-charge distribution on each atom remains the same.

It is of considerable interest to examine the proximity of water molecules around DOX in the solvated model discussed above. To this end, Figure 5a–d show the local atomic positions of water molecules near the N (atom number 12), one of the O atoms (atom number 11), and two C atoms (atom numbers 15 and 24), respectively. These are the atoms that show some changes in the atomic partial charges discussed above, except for N. We found that the shortest distance between an H atom in H_2O and the atoms in DOX is never less than 2 Å. We thus believe that the interaction between water molecules and DOX in the solvated model is weak. As a result, the DOX molecule has only a relatively small overall partial charge of 0.123 e through loss to the surrounding water molecules.

2.3. DOX–DNA Complex

Our ultimate goal was to investigate the electronic structure of DOX in a molecular environment in which it is most important and relevant. Therefore we focused on the interaction of DOX with double-stranded DNA in the DOX–DNA complex (model 3) and calculated the electronic structure and partial charges. The structure of the DOX–DNA complex consists of 932 atoms. We further relaxed the structure obtained from the Protein Data Bank (PDB ID: 1D12) using VASP for better accura-

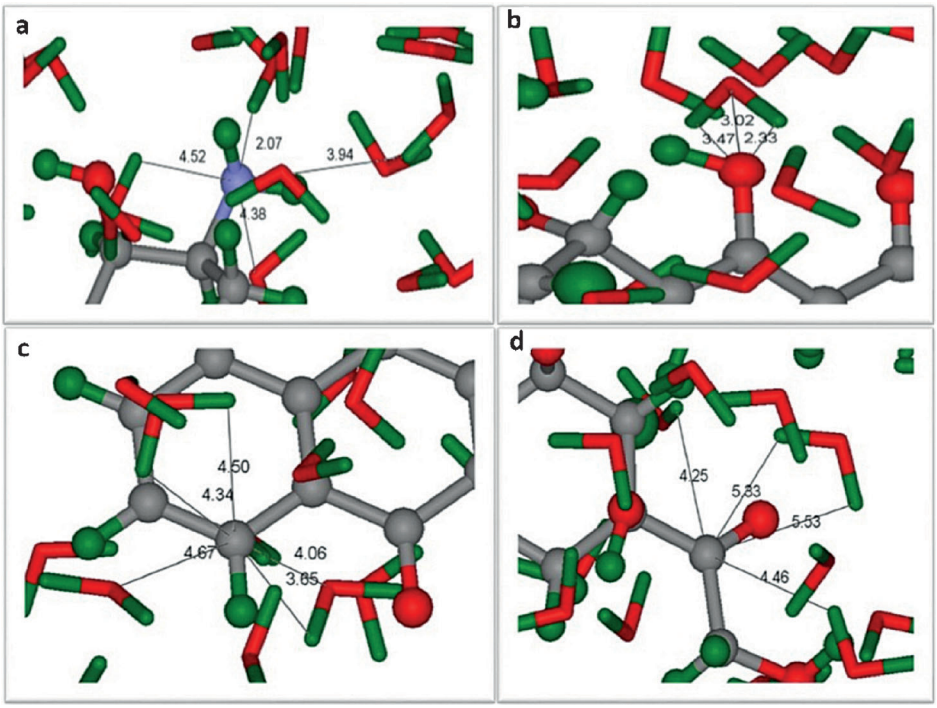

Figure 5. Proximity of H_2O molecules to different atoms in the solvated DOX model. a) N(12), b) O(11), c) C(15), and d) C(24). These selected atoms show slight changes in partial charge when DOX is put in the water box, except for N(12).

cy, as described above. The calculated TDOS of DOX in the DOX–DNA complex (Figure 2c) shows significant differences to those of isolated DOX and solvated DOX. The main differences are the presence of relatively sharp peaks at −23.6, −15, −5.8, and −3.7 eV and the presence of defectlike states in the gap. This is due to the strong interaction of DOX with DNA segments, water molecules, and spermine molecules in a highly complex structure. To better understand these interactions, the TDOS and PDOS of different functional groups in the DOX–DNA complex are shown in Figure 6. The PDOS of water in DOX–DNA is quite different from that of solvated model 2b of Figure 4 because the water molecules in model 3 are all closer to the DOX–DNA complex (see Figure 1c) and thus have stronger electronic interaction. The HOMO–LUMO gap for the DOX–DNA complex is no longer well defined due to the presence of defectlike states above the highest occupied state at 0.0 eV.

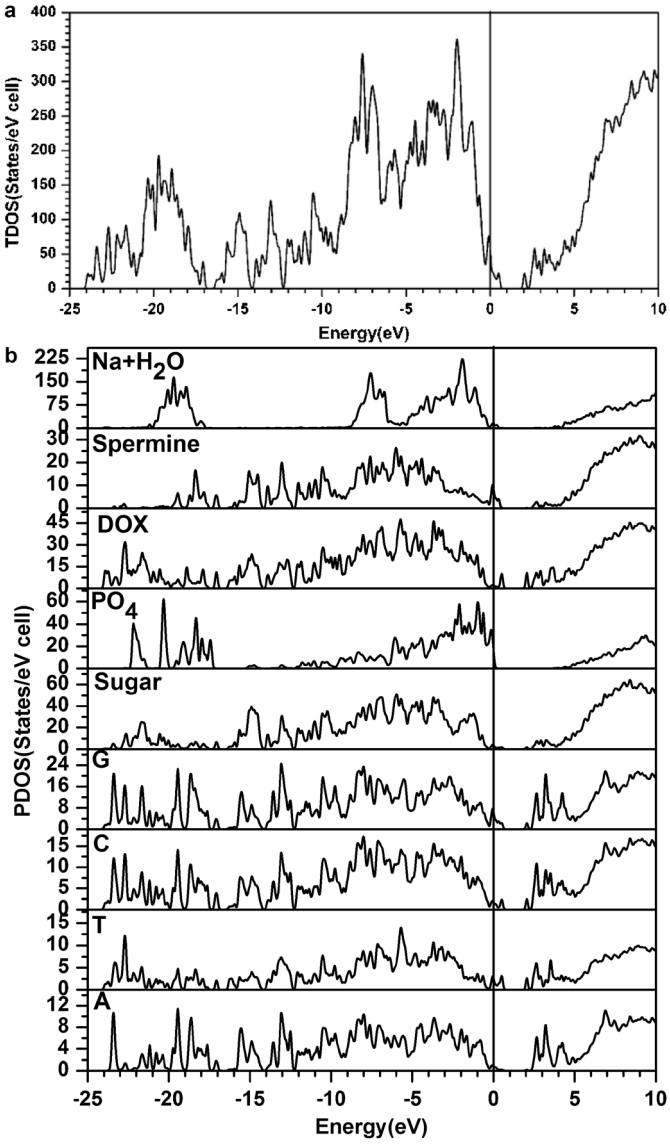

Figure 6. a) Calculated TDOS of DOX–DNA complex. b) Resolution of TDOS into different functional groups. Note that the scales on the *y* axis are not the same for each group.

These states originate from interactions between orbitals of the nucleotide bases of DNA and DOX and, to a lesser extent, water molecules and Na ions. Interestingly, the lower conduction-band region shows three prominent peaks below 5 eV that arise from the DNA bases, very similar to those calculated for various B-DNA models with a different stacking sequence of base pairs.[21]

The atomic partial charges for this large DOX–DNA complex model were calculated in the same way as for the other two models. The distribution of partial charges on the DOX molecule in model 3 is shown in Figure 3c. They are considerably changed due to interaction with DNA. In particular, the partial charge of N becomes more electronegative (−0.89 e) compared with those of model 1 (−0.82 e) and model 2 (−0.81 e). The atomic partial charges for DNA, spermine, water molecules, and Na are shown separately in Figure 7. It is noteworthy that two N atoms of spermine in proximity to the DNA bases actually have positive partial charges, which is quite unusual. This resulted in an overall positive partial charge on spermine due to strong interactions in the DOX–DNA complex. Moreover, one O atom of one of the PO_4 groups also becomes slightly positively charged, possibly due to the presence of water.

The partial charge on each functional group was calculated by adding the atomic partial charges of the constituent atoms. By dividing the partial charge by the solvent-excluded surface area of each functional group, we obtained the surface partial charge density of the functional groups. Table 1 lists the sum of the atomic partial charges on DOX in the three models of different molecular environments, and Table 2 the sums of the atomic partial charges and surface charge density on each functional group in the DOX–DNA complex. Clearly, the partial charge on DOX is now reversed in sign, that is, it is negative (−0.176 e), as opposed to that on solvated DOX in the water box, which is positive (+0.123 e). Table 2 also shows that the DNA bases, the PO_4 group of DNA, and the DOX molecule are all electronegative, whereas the sugar, spermine, and $Na+H_2O$ are all electropositive. In the DNA part, the PO_4 unit is the most electronegative with a partial charge of −10.162 e, whereas the sugars are the most electropositive, with a partial charge of 7.494 e. All DNA bases are electronegative and the absolute magnitudes of their charges follow the order of G > T > C > A. Spermine is also electropositive with a value of 3.609 e, which compensates the negatively charged PO_4 groups when DNA interacts with DOX. $Na+H_2O$, which is another important component in the DOX–DNA complex model, is electropositive with a partial charge of 2.132 e. Its role is primarily to compensate the charge on DNA. Therefore, the DOX–DNA complex without $Na+H_2O$ is a negatively charged cluster with a partial charge of −2.132 e. Thus, a remarkable feature of the DOX–DNA complex is that the solvated DOX switches from electropositive to electronegative on interacting with DNA.

Figure 8 shows the surface partial charge density on the solvent-excluded surface of the model in four different orientations. It shows that the PO_4 moieties are the most negatively charged and sugars the most positively charged molecular

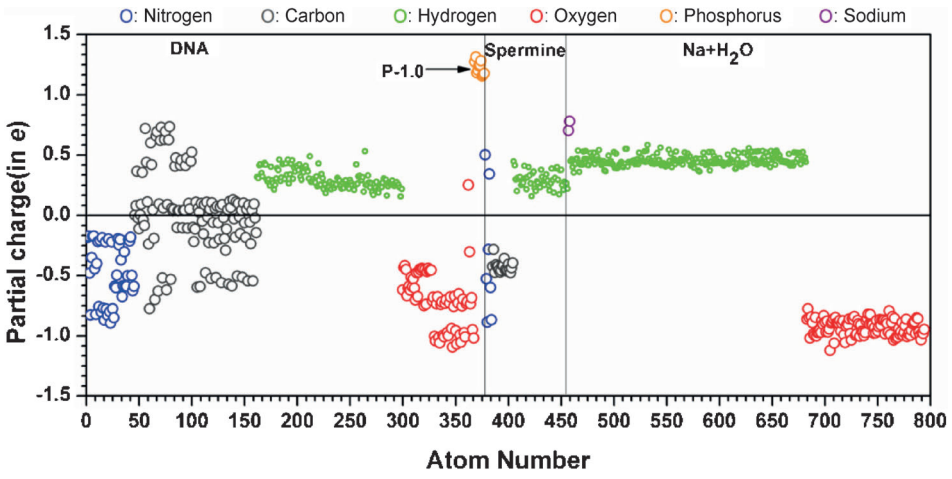

Figure 7. Calculated atomic partial-charge distribution on DNA, spermine and $Na+H_2O$ in the DOX–DNA complex. O red, C gray, N blue, H green, Na violet, P orange. For P, the plotted data ΔQ is decreased by 1 e (marked as P−1.0) for clarity.

Table 2. Sum of the atomic partial charge and surface charge density for different groups in the DOX–DNA complex.

Groups	A	T	C	G	Sugar	PO_4	DOX	Spermine	$Na+H_2O$
$\Sigma(\Delta Q)$ [e]	−0.392	−0.506	−0.484	−1.337	7.494	−10.162	−0.352	3.609	2.132
surface charge density [e nm^{-2}]	−0.256	−0.346	−0.177	−0.421	0.874	−1.917	−0.042	0.676	–

groups. All DNA bases are negatively charged in the order of absolute magnitudes G > T > A > C. These partial charges could have important consequences in quantitative evaluation of electrostatic interactions involving DNA. It is a significant step forward compared with the simplistic description of fixed positive, negative, or zero surface partial charge density commonly adopted in biomolecular research.

2.4. Improvement of DOX–DNA Interaction Energies by NAMD

Using the accurate ab initio partial charges calculated for isolated DOX, solvated DOX, and the DOX–DNA complex, we can estimate the interaction energies in the three cases by utilizing the standard molecular simulation programs as a post-OLCAO calculation to assess the effectiveness and promise of using such a strategy, as outlined in the Methods Section. The planar, rigid chemical structure of DOX does not undergo significant structural changes on DNA intercalation. The structure, including bond lengths and angles, was determined by using ChemDraw3D. This information, together with the partial charges obtained from the OLCAO calculations, was plugged into the NAMD software tool, and the energy between DOX and DNA was then determined by using the NAMD Energy Simulation plugin. The calculated energies are listed in Table 3. These interaction energies give a good indication of how well the DOX molecule fits in the binding pocket when it docks to DNA. Unsurprisingly, the electrostatic interaction of DOX and DNA is much stronger (lower energy) if the partial charges are determined for the full DOX–DNA complex (−121.81 kcal mol^{-1}) rather than dry or sol-

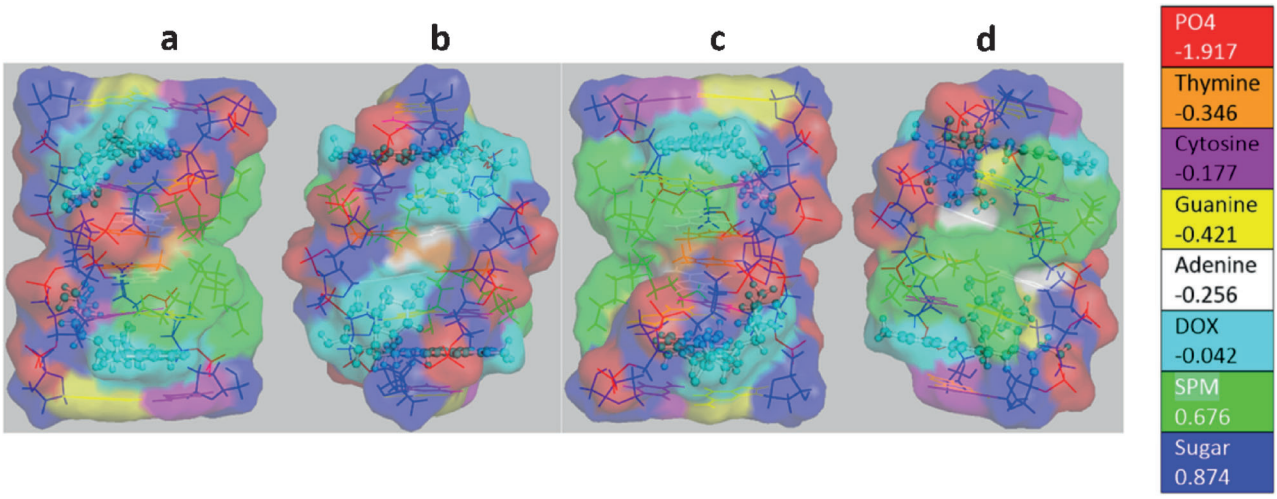

Figure 8. Partial surface charge density on the solvent-excluded surface of the DOX–DNA complex in four different orientations. a) Front view. b) 90° rotation. c) 180° rotation. d) 270° rotation. Partial surface charge densities [e Å^{-2}] are indicated by the color bar. The DOX–DNA complex structure is shown inside the semitransparent surface.

Table 3. Interaction energy [kcal mol^{-1}] of the DOX–DNA complex.

Model	Electrostatic	van der Waals	Total
isolated DOX	+281.949	−8.578	+273.371
solvated DOX	+263.009	−8.578	+254.431
DOX–DNA	−121.81	−9.633	−131.443

vated DOX (281.949 kcal mol^{-1} and 263.009 kcal mol^{-1}, respectively). Furthermore, we observed a more favorable electrostatic interaction of DOX with DNA when the solvent effect on DOX was explicitly considered, although the difference was moderate. The van der Waals interactions were similar for all three molecular environments (−8.578 kcal mol^{-1} for both dry and solvated DOX; −9.633 kcal mol^{-1} for DOX–DNA complex), as determined by NAMD code by using the VMD graphics program. We also determined that the van der Waals interaction energies of the full DOX–DNA complex were slightly higher than those of simpler dry or solvated models. The most significant point of our findings is the importance of accurately optimizing the structural data from the PDB. In a previous separate calculation on the DOX–DNA complex (model 3) using the unrelaxed structure (not shown here), the electrostatic interaction between DOX and DNA is much stronger (+15.642 kcal mol^{-1}), but it still fits the conclusion presented above that the use of ab initio partial charges in the MD codes makes a big difference.

A deeper understanding of molecular interaction is essential for insights into biological systems at the molecular scale. Among the various components of molecular interactions, electrostatics is of special importance because of its long-range nature and its role in polar and/or charged molecules, including water, aqueous ions, proteins, and nucleic acids. In particular, robust models of electrostatic interactions are essential for understanding the solvation properties of biomolecules and the effects of solvation on biomolecular folding, binding, and dynamics.[39] Our results presented above indicate that for accurate quantification of interaction energy in biomolecular complexes one must take fully into account the molecular environment of the interacting molecules. The solvent-stripped molecules cannot be considered to be a valid zeroth-order approximation, since the vicinal solvent layer appears to be closely associated with the biomolecule and contributes in a fundamental fashion to their interaction. Therefore, to obtain more accurate estimates of complexion energy for applications such as computational drug screening, one must include at least one solvation layer in a minimal realistic model. This has been argued for many years on the basis of a different set of thermodynamic measurements.[40] If more information about the molecular environment is known, consideration of these detailed environmental effects on the partial-charge distribution could lead to a much more accurate reflection of the actual interactions of the molecules and a better prediction of the binding site and complexion energy. It was also demonstrated that fully relaxed structural models are of paramount importance. One cannot totally rely on the reported experimental data deposited in data bases such as the PDB. This will remain a great challenge to accurate modeling in computational biomaterials.

3. Conclusions

We studied the electronic structure and partial-charge distribution of doxorubicin in three different model molecular environments, that is, isolated, solvated, and fully intercalated in a DOX–DNA complex. Our results show that solvating water molecules and the proximity of DNA can significantly change the electronic structure and the HOMO–LUMO gap of DOX, as well the distribution of its partial charges. In solvated DOX, both HOMO and LUMO states can be traced to DOX groups themselves. However, in the full DOX–DNA complex, The HOMO–LUMO gap is not well defined and the states near the gap originate from nucleotide bases of DNA. The partial charge of solvated DOX changed drastically from positive to negative in the full DOX–DNA complex. Our calculations clearly showed that, in the DOX–DNA complex, the DNA bases, DOX itself, and PO_4 groups of DNA are all electronegative, whereas the sugar, spermine, and $Na + H_2O$ are electropositive.

In the literature, it has been reported that the influence of molecular environments on the electron density is highly important in complex biomolecules.[41] This is consistent with our findings. Information on the electronic features of DOX in different molecular environments is crucial for its docking and/or complexion with DNA or other biomolecules. The main conclusion of our work is that molecular details of the solvent as well as the details of the interaction geometry matter in determining the stability of DOX complexion. Although the full-scale ab initio simulation of molecular interactions is still beyond our reach, the assessment of solvent effects in the determination of partial charges and molecular surface charge densities that can be obtained from ab initio calculations is an important step towards more adequate modeling of biomolecular interactions that surpasses conventional classical and empirical estimation. In this respect, our ab initio analysis fully supports the often-argued indispensability of the solvent environment for the proper functional integrity of biomolecules.

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Keywords: ab initio calculations · DNA · electronic structure · intercalations · solvent effects

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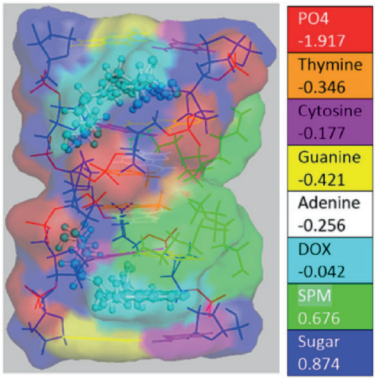
Environmental response: The electronic structure and partial charges of doxorubicin (DOX) in three different molecular environments—isolated, solvated, and intercalated in a DNA complex (see picture)—are studied by ab initio calculations. Solvating water molecules and the proximity to and interaction with DNA have a significant impact on the electronic structure and partial-charge distribution of DOX.



*L. Poudel, A. M. Wen, R. H. French, V. A. Parsegian, R. Podgornik, N. F. Steinmetz, W.-Y. Ching**

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Electronic Structure and Partial Charge Distribution of Doxorubicin in Different Molecular Environments

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