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REGULAR ARTICLE

A three-point method for evaluations of AMBER force field parameters: an application to copper-based artificial nucleases

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Abstract We present the theoretical evaluation of new AMBER force field parameters for 12 copper-based nucleases with bis(2-pyridylmethyl) amine, 2,2′-dipyridylamine, imidazole, *N*,*N*-bis(2-benzimidazolylmethyl) amine and their derivative ligands based on first-principles electronic structure calculations at the B3LYP level of theory. A three-point approach was developed to accurately and efficiently evaluate the force field parameters for the copper-based nucleases with the ligands. The protocol of RESP atomic charges has been used to calculate the atomic charge distributions of the studied copper-based nucleases. The evaluated force field parameters and RESP atomic charges have been successfully applied in the testing molecular mechanics calculations and molecular dynamics simulations on the nucleases and the nuclease–DNA complexes, respectively. It has been demonstrated that the developed force field parameters and atomic charges can consistently reproduce molecular geometries and conformations in the available X-ray crystal structures and can reasonably predict the interaction properties of the nucleases with DNA. The developed force field parameters in this work provide an extension of the AMBER force field for its application to computational modeling and simulations of the copper-based artificial nucleases associated with DNA.

Keywords AMBER force field parameter · Three-point method · Artificial metallonuclease · Copper complex

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1 Introduction

During the past decades, transition metal complexes have drawn considerable attention in rational design of novel artificial metallonucleases because of their potential application in anticancer drugs and effective chemotherapeutic agents for many diseases [1–4]. Among the various transition metal complexes investigated to date, copper complexes are the extensively studied artificial nucleases since they have shown DNA cleavage activity under physiological conditions in biological systems [5–8]. In developing efficient copper nucleases, it is of vital importance to identify the steric requirements of DNA binding interactions [9]. Many factors, including the size and shape of copper nucleases, coordination environment of copper center, ligand structure and nuclearity of copper complexes, can be modified in the interaction studies of nucleases with DNA in order to improve their nuclease activities [10, 11]. Various copper nucleases have been investigated in the past two decades [12]. For instance, a great number of copper nucleases [13, 14] with bis(2-pyridylmethyl) amine (BPA) [15], *N*, *N*-bis(2-benzimidazolylmethyl) amine (IDB) [16], and their derivative ligands [17] have attracted much attention due to their synthetic accessibility, low molecular weight, and efficient DNA-binding/cleavage ability [18]. Recent advances in this field have provided important insights into the mechanism of DNA cleavage [19, 20] and

the DNA-cleaving reactivity for these types of copper-based complexes [21, 22]. It was proposed that in the presence of reactive oxygen species, such as H_2O_2, O_2, and reducing agents, copper nucleases as oxidative cleavage agents can attack the sugar or base moieties of DNA, and ultimately achieve DNA strand scission [7, 13, 23]. However, the mechanisms of copper nucleases damaging DNA are complicated, and several issues in the mechanisms remain unresolved [24, 25]. Even though some valuable experimental results demonstrated the DNA-cleavage activity of some copper nucleases [9, 15], the investigations on nuclease–DNA binding modes and cleavage mechanisms of DNA by nucleases at molecular level are still quite limited so far.

It is important for improving the DNA-cleavage activity and for rationalizing the experimental results to better understand the structures and properties of copper nucleases binding to DNA and the mechanisms of DNA-cleavage by the copper nucleases. Molecular mechanics (MM) calculations and molecular dynamics (MD) simulations provide powerful tools for studying the structures, conformations, and interactions of the biological macromolecules. By employing an analytic energy function, that govern all relevant molecular energetic, structural, and dynamic properties [26], of atomic coordinates (i.e. a force field), MM and MD studies can provide proper solutions and guidance to various biological problems, such as DNA-binding/cleavage mechanisms. However, the accuracy of the MM or MD calculations depends greatly on the force field parameters used in the MM or MD calculations [27]. Many force fields, such as the AMBER [28–30], CHARMM [31, 32], UFF [33], GROMACS [34], COMPASS [35] and MOMEC [26], have been developed extensively for simulating the structures and conformational energies of normal nucleic acids, proteins, and organic molecules in the past decades. Particularly, the AMBER force field parameterization, first published in 1984, was developed by Kollman et al. [36]. However, with the development of computational simulations on some new types of molecules, additional force field parameters are still desired. For instance, AMBER force field parameters for phosphorothioate nucleic acids, various polyphosphates, and modified nucleosides have been developed very recently [37].

In general, the Generalized Amber Force Field (GAFF) is a good choice for molecules once all atom types of the target molecule are available in GAFF. However, the atom types of most transition metals are not defined in the current version of GAFF [29]. One has to evaluate the force field parameters for these atoms in order to perform MM or MD calculations on the molecules containing transition metal atoms. On the other hand, the selection of a proper approach applied to develop AMBER force field parameters is somewhat a tricky task. The method of fitting the potential energy surface is the most extensively applied one among many methods of force field parameter calculations [38–42]. It is a common practice to determine the force field parameters for each geometric parameter (such as bond length, bond angle, or dihedral angle) through fitting the force field-based energy profiles to those calculated by performing quantum mechanical (QM) calculations on various geometries of the molecular system around a local-minimum geometry.

Unfortunately, such a commonly used method is computationally demanding and is very time-consuming in the development of force field parameters for a molecule with a large number of geometrical parameters. Theoretically, a more efficient alternative may be developed to evaluate the force field parameters. For example, we can calculate the secondary derivative of molecular energy with respect to a bond length by calculating the molecular energies of three geometries at adjacent bond lengths. The same approach can be used for a bond angle. This alternative approach (referred as a three-point approach below) of the force field parameter development is expected to be as accurate as the above-mentioned commonly used approach, but will be considerably more efficient in practical computations.

Despite the need of MM/MD studies on copper-based nucleases, force field parameters for these types of molecules are rarely available [26]. We have recently developed two sets of AMBER force field parameters related to the copper center with the BPA and IDB ligands by using the energy profile fitting method [43]. However, to the best of our knowledge, no attempt has been made on the parameterization for more copper-based nucleases so far. Here, we report the evaluation of the AMBER force field parameters for 12 representatives of four typical classes of copper-based nucleases that cover most types of the copper-based nucleases involved in the redox cleavage feature of DNA [9, 15, 16, 22, 44–48], i.e. six nucleases with BPA/(2,2′-dipyridylamine) DPA/derivative ligands, three nucleases with imidazole (IM)/derivative ligands and three nucleases with IDB/derivative ligands (as shown in Fig. 1), by using the three-point approach. To test the reliability of the developed force field parameters, MM calculations on the nucleases and MD simulations on nuclease bound to the minor groove of DNA with ten base pairs have also been performed.

2 Computational methods

2.1 Parameterization strategy

The basic equation of the AMBER force field [28] for the total energy of a molecular system is

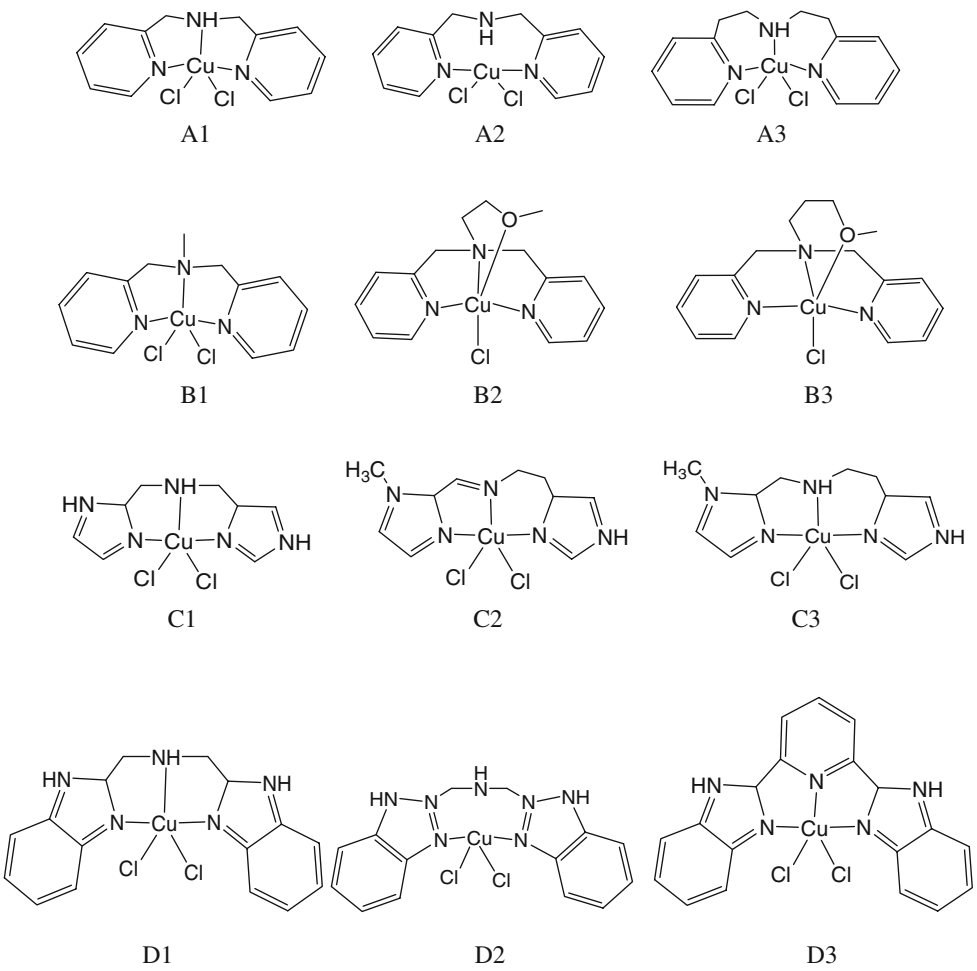

Fig. 1 Components of 12 studied copper-based nucleases

$$E = \sum_{\text{bonds}} K_{\text{r}}(r - r_{\text{eq}})^2 + \sum_{\text{angles}} K_{\theta}(\theta - \theta_{\text{eq}})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] + \sum_{i<j} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\varepsilon R_{ij}}\right] \quad (1)$$

where the four constituent terms describe bond stretching, angle bending, torsion, and nonbonded interactions, respectively. The symbols of r, r_{eq}, and K_{r} are, respectively, bond length, equilibrium value, and the force constant for the bond stretching; θ, θ_{eq}, and K_{θ} are, respectively, bond angle, equilibrium value, and the force constant for the angle bending terms. The third term is the energy contribution from torsional motion, in which ϕ, V_n, n, and γ are, respectively, the torsion angle, force constant, the periodicity, and the phase angle. The last term describes nonbonded interactions. To calculate the energy of a molecule at a given geometry, the constant parameters in Eq. 1 are required.

The AMBER force field parameters for 12 representatives of four typical classes of copper-based nucleases with BPA, DPA, IM, IDB and their derivative ligands are evaluated by following this basic equation. In this study, only some force field parameters around the copper center, namely the bond lengths and bond angles around the copper for each studied nuclease, are of interest. The molecular geometries and energies were calculated with the hybrid density functional B3LYP [49] method as implemented in the GAUSSIAN 03 software [50]. In all calculations, we have used the double-ζ basis set of Schäfer et al. (62111111/33111/311) [51], enhanced with diffuse p, d, and f functions with exponents 0.174, 0.132, and 0.39 (called DZ*pdf*) for copper atoms [52]. The 6-31G** basis set was employed for other atoms [53]. The equilibrium structures for these studied compounds were fully optimized to obtain the equilibrium parameters consistent well with the experimental data [9, 15, 16, 22, 44–48]. In order to verify a suitable DFT method for these studied systems, the newer DFT method, M05 developed by Truhlar et al. [54–62] have also been employed to optimize some structures of the studied complexes with 6-311G** basis set [63]. The force constants of bond length (K_{r}) and bond angle (K_{θ}) are evaluated by using a three-point method.

It is clear that if we assume all the terms in Eq. 1 are adiabatic then we have

$$K_r = \frac{1}{2}\frac{\partial^2 E}{\partial r^2}; \quad K_\theta = \frac{1}{2}\frac{\partial^2 E}{\partial \theta^2} \tag{2}$$

The task for the calculation of force field parameters is converted to evaluate the secondary derivative of energy with respect to bond length or bond angle. The three-point method is in fact a computationally simplest numerical differential method to estimate force constants in which energies at only three geometries of adjacent bond lengths or bond angles are required. For instance, the secondary derivative of molecular energy with respect to a bond length at equilibrium r_{eq} is

$$\frac{\partial^2 E(r_{eq})}{\partial r^2} \approx \frac{1}{(\Delta r)^2}\left[E(r_{eq}+\Delta r) + E(r_{eq}-\Delta r) - 2E(r_{eq})\right] \tag{3}$$

where Δr is the increment of bond length r. The secondary derivative of energy with respect to a bond angle is calculated in the same way. Thus, the secondary derivative of molecular energy is obtained by calculating energies at three geometries in which the bond length or bond angle are changed by a small increment around the equilibrium value, while the rest of the geometry is fully relaxed. Furthermore, we compared force field parameters evaluated by using the three-point method with those obtained by using a previously reported iterative least-square method [43] which is based on the energy profiles fitting to the DFT-calculated energy profiles [41, 64–68].

The electrostatic potential representing atomic charges for the studied copper nucleases based on the optimized geometries were calculated at the HF/6-31G** [53] +DZ*pdf* [51, 52] level of theory. Furthermore, the well-known standard restrained electrostatic potential (RESP) charge approach [69, 70] was used to determine the atomic charges, as the RESP charge approach is one of the most widely used and well behaved types of atomic charges used in MM and MD simulations.

2.2 MM and MD calculations

To validate the determined force field parameters and atomic charges, MM calculations of copper nucleases alone and MD simulations of copper nuclease–DNA complexes for some copper nucleases, i.e. B1, C3, and D1 (see Fig. 1), were carried out. The MM potential energy functions, which were built by the evaluated force field parameters and atomic charges in this work, were employed to minimize the energies of these copper nucleases. The docked conformations of nucleases and DNA for MD simulations as the initial structures were estimated by using the AUTODOCK 3.0 program [71]. In addition to the force field parameters around the copper center evaluated in this work, other force field parameters unassociated with Cu ion in each nuclease were generated by using the ANTECHAMBER module of AMBER 9 program [72]. The systems were explicitly solvated by using the TIP3P water potential inside a box large enough to ensure the solvent shell extended to 10 Å in all directions of each system studied. For the equilibration of the complex, the following procedures were carried out. The energy minimizations of copper nuclease–DNA complexes with 12,500 steps were first performed to remove unfavorable contacts, which involved four step simulations with the gradually decreased restraints. Then the complexes were subjected to 120 ps of heating process from 0 to 300 K, followed by 200 ps unrestrained MD simulations for the purpose of equilibration. Subsequently, the unrestrained production MD runs of 10 ns were performed at normal pressure (1 atm.) in the NPT ensemble for each system. Nonbonded interactions were truncated using the spherical cutoff 10 Å. All MD simulations were performed by using the SANDER module of AMBER 9 program package [72]. Visualizations of the systems were done via VMD software [73].

3 Results and discussions

3.1 Copper nuclease structures

For each of the studied nucleases, as can be observed in Fig. 1, the central copper ion lies in an N/Cl/O coordination environment composed of three nitrogen atoms from the surrounding ligands and two chloride ions or one chloride atom/one oxygen atom, except for A2 and D2 complexes composed of only two nitrogen atoms from the planar ligands and two chloride ions (see Fig. 1). The spatial conformations of the studied complexes are close to the square pyramidal structures due to Jahn–Teller effects. The ligand field molecular mechanics (LFMM) method has been developed by Deeth et al. [74, 75] for solving the Jahn–Teller effect. Especially, more recently, the LFMM model has been successfully applied to a range of Cu(II) complexes [76–81]. Based on the LFMM theory, the optimized geometries for these nucleases represent two kinds of coordination environments, e.g. three/two N atoms and two/one Cl counterions coordinating to the copper center, which reproduces the characteristics of corresponding experimental structures. The optimized geometric parameters for selected bond lengths and angles are shown in Tables 1 and 2 along with the available experimental data. Obviously, from Tables 1 to 2, most optimized geometrical parameters of the copper nucleases agree well with the available data determined by X-ray diffractions,

Table 1 Optimized structural parameters by B3LYP and M05 methods for A and B classes of copper-based nucleases along with X-ray data (bond Å and angle degree)

	A1		A2	A3			B1			B2		B3	
	Calc	Expt[a]	Calc	Calc	Calc[b]	Expt[c]	Calc	Calc[b]	Expt[d]	Calc	Expt[e]	Calc	Expt[d]
Cu–n3	2.076	1.993	–	2.107	2.079	2.067	2.099	2.064	2.035	2.136	2.060	2.136	2.029
Cu–nd	2.027	1.987	2.065	2.049	2.044	2.025	2.022	2.011	1.981	2.032	1.989	2.028	1.975
Cu–Oa	–	–	–	–	–	–	–	–	–	2.380	2.381	2.401	2.398
ca–ca	1.393	1.379	1.394	1.393	1.386	1.374	1.393	1.387	1.383	1.393	1.352	1.393	1.392
Ca–nd	1.349	1.347	1.343	1.351	1.346	1.342	1.348	1.342	1.345	1.347	1.346	1.346	1.372
c3–n3	1.481	1.477	1.462	1.493	1.484	1.487	1.485	1.473	1.482	1.490	1.487	1.494	1.506
nd–Cu–nd	162.3	165	154.4	163.7	167.5	176.7	162.5	164.1	164.5	157.6	161.0	159.9	164.1
nd–Cu–n3	81.3	82.9	77.5	88.9	89.4	89.8	81.7	82.2	82.3	81.9	83.5	82.0	82.9
ca–nd–Cu	120.5	120.5	120.0	120.2	120.6	120.3	120.0	120.1	120.3	120.0	126.4	120.0	121.4
c3–n3–Cu	108.9	109.9	101.6	114.1	114.1	114.5	106.7	106.5	108.6	106.2	111.1	108.0	109.1
n3–Cu–Oa	–	–	–	–	–	–	–	–	–	78.6	79.8	91.2	93.5
nd–Cu–Oa	–	–	–	–	–	–	–	–	–	95.9	94.9	96.2	94.0
c3–Oa–Cu	–	–	–	–	–	–	–	–	–	114.9	110.6	122.9	121.7

[a] Taken from Refs. [9, 15, 44]

[b] M05 method

[c] Taken from Ref. [91]

[d] Taken from Ref. [92]

[e] Taken from Ref. [48]

even though the X-ray data employed to compare with the calculated values cause a certain approximation. By examining the deviations of the optimized geometrical parameters from the X-ray structures, the computed bond lengths and bond angles for these complexes are larger by 1.41 and 0.87% (in average), respectively, than the corresponding experimental data [9, 15, 16, 22, 44–48]. For example, the optimized average distances between the Cu ion and Nd coordinate atoms of the planar ligands or N3 atom at the linking region of two planar ligands are around 2.02 or 2.13 Å compared to average value of 1.99 or 2.03 Å in the crystal structures. The optimized angles, relating to the copper center, of 157°–169° for nd–Cu–nd angle and of 77°–89° for nd–Cu–n3 angle agree well with the respective values of 130°–177° and 79°–90° observed in the X-ray structures [9, 15, 16, 22, 44–48]. The optimized geometries of the studied copper complexes are all in good agreement with the available experimental values [9, 15, 16, 22, 44–48].

The geometries of A3, B1, C3 and D1 complexes as the representatives of the four classes of the complexes have also been optimized by DFT/M05 method. The corresponding geometric parameters, the corresponding dipole moments and bond dissociation energy have been shown in Tables 1, 2 and 3, respectively. The calculated results show that the geometric parameters of the studied four complexes calculated by both M05 and B3LYP methods are closed to the experimental data (see Tables 1, 2). The bond dissociation energy of 102.4 kcal mol^{-1} for c3–ca bond of B1 complex obtained by M05 method are slightly more close to the experimental value of 99.8 kcal mol^{-1} [82] than that of 94.4 kcal mol^{-1} by B3LYP method (see Table 3). The calculated dipole moments of the studied complexes are similar (see Table 3). All results demonstrate that the DFT/M05 method is a greatly improved method for the calculations of Cu-based complexes. However, in order to save computational resource, the B3LYP method has been employed for the evaluations of force field parameters.

3.2 RESP charges

In MM calculations and MD simulations, atomic charges affect greatly the computational results concerning the properties related to molecular energies and geometries. Therefore, various techniques for atomic charge calculations by using quantum chemical methods have been proposed previously [69, 70, 83, 84]. The RESP charges, generated by the electrostatic potentials calculated by Merz–Kollman method, have been calculated for the atomic charges of all studied copper nucleases. The defined atom types and calculated RESP atomic charges for 12 studied copper nucleases are shown in Fig. 2 and Table 4, respectively. The previous studies on DNA cleavage mechanisms suggested that the DNA molecule and copper nucleases are the respective electron–donor and electron–

Table 2 Optimized structural parameters by B3LYP and M05 methods for C and D classes of copper-based nucleases along with X-ray data (bond Å and angle degree)

	C1	C2		C3			D1			D2	D3
	Calc	Calc	Expt[a]	Calc	Calc[b]	Expt[a]	Calc	Calc[b]	Expt[c]	Calc	Calc
Cu–n3 (nd′)	2.175	2.108	2.063	2.181	2.136	2.018	2.171	2.129	2.025	–	2.027
Cu–nd	1.969	1.998	2.010	1.981	1.976	1.960	2.007	1.998	1.984	2.089	2.057
ca–ca	1.368	1.376	1.354	1.368	1.363	1.347	1.400	1.393	1.382	1.401	1.401
ca–nd	1.381	1.374	1.377	1.383	1374	1.386	1.399	1.391	1.391	1.372	1.384
c3–n3 (nd′)	1.484	1.459	1.466	1.485	1.476	1.481	1.478	1.468	1.497	–	–
nd–Cu–nd	159.3	167.5	161.7	168.9	171.3	171.3	158.9	160.2	158.9	130.1	156.9
nd–Cu–n3 (nd′)	80.3	85.1	85.0	85.3	85.9	86.5	79.5	80.2	79.7	–	78.4
ca–nd–Cu	126.6	133.4	134.8	132.2	132.3	133.3	138.5	139.4	140.3	126.5	140.1
c3–n3 (nd′)–Cu	109.3	125.1	125.0	113.6	113.4	113.2	109.5	109.8	112.9	–	–
cc–nd–Cu	125.5	119.1	118.8	120.3	120.4	119.9	114.9	114.2	114.0	–	113.1

[a] Taken from Refs. [45, 46]

[b] M05 method

[c] Taken from Ref. [16]

Table 3 Dipole moments (Dipole) (Debye) and bond dissociation energies (BDE) (kcal mol^{-1}) calculated by B3LYP and M05 methods

	A3	B1	C3	D1		B1
Dipole (B3LYP)	7.37	7.73	8.26	12.27	BDE (B3LYP)	94.4
Dipole (M05)	7.96	8.31	8.89	12.88	BDE (M05)	102.4

acceptor [14, 85]. As expected, the copper atom charges for all studied nucleases are positive (as shown in Table 4), which will facilitate to cleave the DNA molecule.

3.3 Force field parameters

Generally, the force field parameters for normal molecules [26, 27, 39], such as amide acids, nucleotides, and normal organic molecules, can be determined only by the atom types, and ignore the environment characteristics. However, it is a key feature of force field parameter evaluations for transition metal compounds that the force field parameters are uniquely determined by the characteristics of both metal center and coordinated ligands through the geometric representations [64]. The 12 compounds of four classes of copper nucleases characterize each center copper ion coordinated by two N atoms of two pyrimidines at the equatorial plane and one N atom of corresponding linking region for each compound of A and B classes, i.e. A1, A2, A3, B1, B2, and B3, similarly by two N atoms of two imidazoles and one N atom of corresponding linking region for each compound of C class, i.e. C1, C2, and C3, and by two N atoms of two IDB ligands and one N atom of corresponding linking region for each compound of D class,

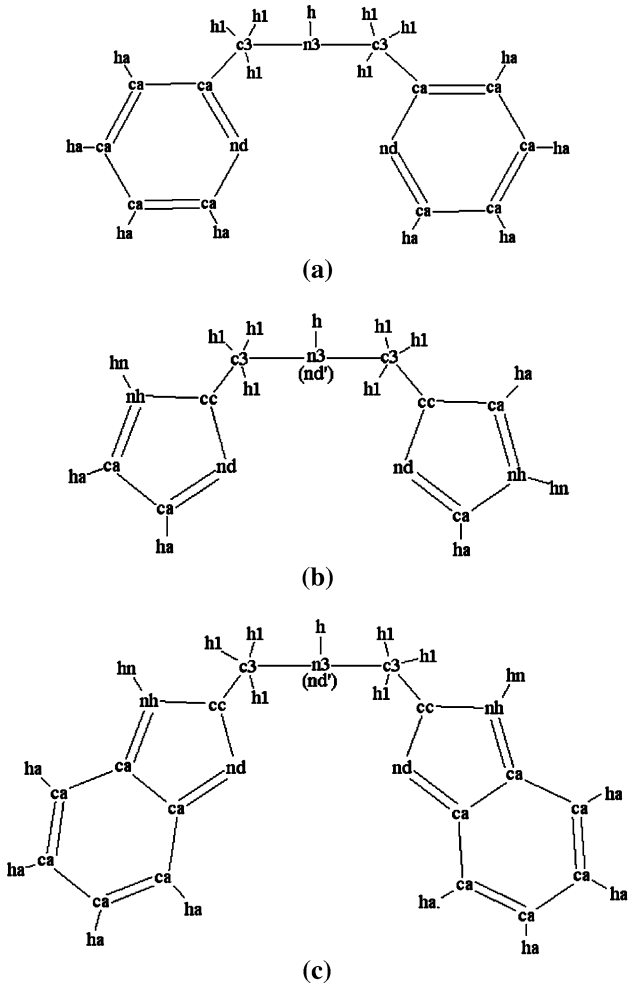

Fig. 2 Defined atom types of the ligands in the studied copper nucleases

Table 4 RESP charges of main atoms for 12 studied copper nucleases

Atom type	A1	A2	A3	B1	B2	B3
Cu	0.3129	0.3943	0.5073	0.1776	0.0218	0.3014
n3	−0.0167	−0.5326	−0.2929	0.3058	0.1283	−0.0047
nd	0.1570	0.2726	0.0140	0.1739	0.1839	0.0243
ca	−0.0949	−0.1211	−0.0821	−0.1057	−0.0725	−0.0436
c3	−0.0661	0.0655	−0.0038	0.0874	−0.0664	−0.0885
ha	0.1694	0.1536	0.1590	0.1759	0.1426	0.1256
h1	0.1042	0.0885	0.0736	0.0054	0.0867	0.0864
h	0.1889	0.3070	0.2421	–	–	–
Oa	–	–	–	–	−0.1785	−0.1732

Atom type	C1	C2	C3	D1	D2	D3
Cu	0.4496	0.4536	0.5158	0.2486	0.5168	0.0053
n3 (nd′)	−0.1098	0.0846	−0.1422	0.0245	−0.0438	0.4449
nd	−0.0378	−0.1317	−0.0970	0.1879	−0.1690	0.1961
nh	−0.2370	−0.0434	−0.0286	−0.1122	−0.1774	−0.2075
ca	−0.1180	−0.1214	−0.1967	−0.1060	−0.0232	−0.1077
c3	0.0840	−0.2348	−0.0277	−0.1921	−0.4097	–
cc	0.0047	0.2019	0.1190	0.0442	–	0.1245
ha	0.1993	0.2148	0.2391	0.1512	0.0758	0.1628
hn	0.3705	0.2139	0.2490	0.3201	0.2733	0.3821
h1	0.0707	0.1509	0.0852	0.1413	0.2000	–
h	0.1948	–	0.2101	0.1843	0.2079	–

i.e. D1, D2, and D3, besides the two Cl counterions for each compound (see Fig. 1). The three-point method described above was employed to evaluate the AMBER force field parameters for the 12 copper nucleases. It is well known that the harmonic-oscillator approximations, which plays an important role in the AMBER force field parameter calculations, will not hold as the values of bond lengths or angles are far from their equilibrium values. Taken it into account, the examination of appropriate increment for the bond lengths and angles is important for a successful application of the three-point method. Figure 3 shows the changes of ΔK_r (K_r at a given increment Δr minus the smallest K_r among all the K_r at different Δr) and ΔK_θ (K_θ at a given incremental $\Delta\theta$ minus the smallest K_θ among all the K_θ at different $\Delta\theta$) with the changes of Δr and $\Delta\theta$, respectively. It can be seen from Fig. 3 that the increments range from 0.01 to 0.1 Å for bond stretching or from 1° to 5° for angle bending can be reasonably used in the three-point method due to the consistence of the evaluated force field parameters for the studied examples. Therefore, the rational increments of ±0.1 Å and ±1° for bond stretching and angle bending, respectively, around the equilibrium values were employed to the present calculations according to the harmonic oscillation approximation.

The force field parameters for the 12 compounds of four classes of the copper nucleases have been evaluated by using the three-point method and summarized in Tables 5 and 6, along with the data including the similar values with the same atom types but different ligand environments [9, 15, 16, 22, 44–48] as well as the data from our previous studies on B1 and D1 compounds [43]. As expected, the force field parameters with same atom types for each compound present the certain differences due to the connected different ligand environments. However, as one can see from Tables 5 and 6, most of the evaluated parameters for the same atom types of the studied systems are consistent with the similar data evaluated by the energy profile fitting method, such as copper–imidazole and copper–pyrimidine types. For example, the force field parameters of 108.352 kcal mol^{-1} Å^{-2} and 20.043 kcal mol^{-1} rad^{-2} for the Cu–Nd bond stretching and nd–Cu–nd angle bending of C2 complex are consistent with 101.1 kcal mol^{-1} Å^{-2} and 24.6 kcal mol^{-1} rad^{-2}, respectively, evaluated by the energy profile fitting method [68]. Particularly, the evaluated parameters for compounds B1 and D1 reproduce mostly our previous results calculated by using the energy profile fitting method [43]. In addition, the evaluated force constant of 110.147 kcal mol^{-1} Å^{-2} for the Cu–Nd bond stretching of C1 complex is comparable with 182.9 kcal mol^{-1} Å^{-2} estimated by UFF [33]. On the other hand, it has been examined that the evaluation of these parameters by using the new method can save the computational time by the percentage of about 60 with respect to using the energy profile fitting method. Considering the sensitivity of force field parameters upon the configuration changes of copper center region included by ligand environments [43, 64], the parameters around copper center can fluctuate in certain ranges. For example, the previous studies reported in literature demonstrate some reasonable variation ranges of the force field parameter values for some atom types around the copper center, such as K_r values of 33–101 kcal mol^{-1} Å^{-2} for Cu–N3, 59–220 kcal mol^{-1} Å^{-2} for Cu–Nd, and K_θ values of 21–63 kcal mol^{-1} rad^{-2} for ca–nd–Cu [41, 64, 66–68]. Some parameters presented in this work are comparable with these reported results with a reasonable deviation due to the different coordinated environments around the copper center. Namely, the current average parameter values of 65.635, 88.609 kcal mol^{-1} Å^{-2} and 66.009 kcal mol^{-1} rad^{-2} for Cu–N3, Cu–Nd and ca–nd–Cu match the reported ranges of 33–101, 59–220 kcal mol^{-1} Å^{-2} and 21–63 kcal mol^{-1} rad^{-2} [41, 64, 66–68], respectively. Because the dihedrals connecting the copper ion for each compound are rigid, their corresponding parameters can generally use the standard values of AMBER program.

To address the correlation of the force field parameters with the ligand environment of central Cu ion, the

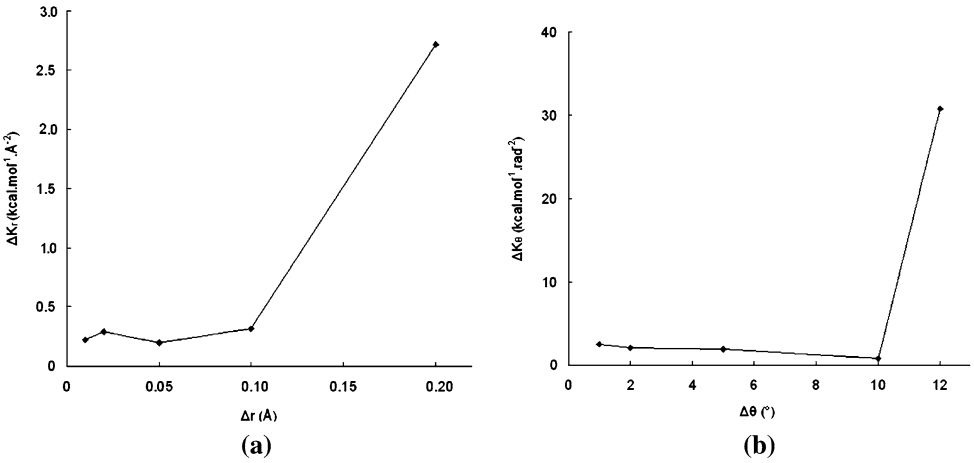

Fig. 3 Changes of relative force field parameters (ΔK_r and ΔK_θ) along with increments of bond stretch (**a**) and angle bend (Δr and $\Delta\theta$) (**b**). ΔK_r (ΔK_θ) is the difference between K_r (K_θ) at a given increment Δr ($\Delta\theta$) and the smallest K_r (K_θ) among all the K_r (K_θ)

Table 5 Evaluated force field parameters with respect to copper center for A and B classes of copper-based nucleases along with similar data (K_r kcal mol^{-1} Å^{-2}; K_θ kcal mol^{-1} rad^{-2})

	A1	A2	A3	B1	B1[a]	B2	B3	Literature[b]
K_r								
Cu–n3	83.44	–	61.628	76.848	64.396	70.451	67.818	33–101
Cu–nd	94.478	60.485	70.344	96.668	73.300	89.260	93.361	59–220
Cu–Oa	–	–	–	–	–	20.309	19.202	36
K_θ								
nd–Cu–nd	49.852	38.309	37.492	43.620	42.432	21.012	24.050	15–42
nd–Cu–n3	80.484	–	68.392	82.554	113.397	82.297	90.125	
ca–nd–Cu	57.783	82.297	56.444	51.529	85.470	50.006	49.903	21–85
c3–n3–Cu	36.524	–	55.002	63.242	67.216	41.921	39.969	
n3–Cu–Oa	–	–	–	–	–	54.281	30.179	
nd–Cu–Oa	–	–	–	–	–	8.755	8.549	
c3–Oa–Cu	–	–	–	–	–	8.652	17.098	

[a] Taken from Ref. [43] of our previous work

[b] Taken from Refs. [41, 64–68]

characteristics of parameters associated with the structural features for these studied compounds present that the force field parameters of Cu–Nd bond length are generally larger than those of Cu–N3 because the average Cu–N3 bond length is longer by 0.1 Å than the average Cu–Nd bond length, and that the force field parameters of nd–Cu–nd bond angle versus nd–Cu–n3 present the similar rule. As expected, the force field parameters are influenced by small but significant structure changes through the redistribution of electron density caused by the variation of coordination ligands, which has been reported by the Comba group [64]. That is to say, the change of ligand environment around the central copper ion induces the variation of electronic density of coordination atoms upon copper center and, therefore, can cause the change of corresponding force field parameters.

3.4 Parameter validation

To validate the evaluated force field parameters and RESP charges in this work, the MM calculations and MD simulations for B1, C3, and D1 nucleases, as the tested examples, were carried out using the AMBER9 package. The force field parameters related to the copper ion region for each studied compound have been set up by using the currently evaluated parameters. The rest of the force field parameters for the atoms far from the copper center are taken from those of parm99 and gaff force fields in the AMBER9 program. The initial structures for the MM calculations are taken from the X-ray structures [15, 16, 45]. The root-mean-square deviations (RMSDs) of the MM calculations with respect to the X-ray data for the studied compounds have been examined and shown in Table 7. It

Table 6 Evaluated force field parameters with respect to copper center for C and D classes of copper-based nucleases along with similar data (K_r kcal mol^{-1} Å^{-2}; K_θ kcal mol^{-1} rad^{-2})

	C1	C2	C3	D1	D1[a]	D2	D3	Literature[b]
K_r								
Cu–n3(nd′)	54.791	62.663	52.733	54.367	62.100	–	110.636	33–101
Cu–nd	110.147	108.352	105.782	95.755	76.202	60.452	78.228	59–220
K_θ								
nd–Cu–nd	39.140	20.043	19.982	43.878	37.993	44.970	61.748	15–42
nd–Cu–n3(nd′)	80.288	61.439	57.886	92.494	97.359	–	102.897	
ca–nd–Cu	44.032	43.878	49.131	51.603	62.586	32.754	56.255	21–85
c3–n3(nd′)–Cu	36.668	47.277	42.642	69.010	46.327	–	–	–
cc–nd–Cu	61.594	64.478	44.753	71.688	77.281	–	46.371	38–77

[a] Taken from Ref. [43] of our previous work

[b] Taken from Refs. [41, 64–68]

can be seen from Table 7 that the average RMSD values of the MM calculated atomic positions from those in the X-ray crystal structures are only 0.12, 0.16, and 0.27 Å for B1, C3, and D1 nucleases, respectively, which suggests that the MM energy minimization using our developed new force field parameters can satisfactorily reproduce the experimental structures.

Taking the proposed mechanisms of DNA strand scission [3, 86, 87] and the popular binding mode [88] into account, the most reasonable conformation model from the 500 docked conformations by AUTODOCK 3.0 program [71] for each nuclease–DNA complex, B1 (C3 or D1) +DNA [15, 16, 45], was selected as the initial structure for MD simulation. After the MD simulation of 10 ns, the average RMSD values of all backbone atoms of nuclease–DNA complex, the DNA molecule, and the nuclease with respect to its starting structure for each nuclease–DNA complex have been analyzed. The corresponding results obtained for the C3 nuclease–DNA complex are shown in Fig. 4. The average RMSD values of the simulated atomic positions from the X-ray data during the MD simulations for three nucleases are given in Table 7. It can be seen from Fig. 4 that this complex (C3 nuclease–DNA) was well equilibrated after 200 ps MD running and remained stable during the simulation in the explicit solvent. The mean-square fluctuation of the complex with respect to the initial structure correlated with temperature factors is small, which means that the structures of C3 and DNA agree well with the experimental X-ray structures during the simulation [45, 46]. Particularly, the C3 structure exhibited a much smaller flexibility than that of DNA duplex, e.g. the average RMSD values of backbone atoms were 0.17 and 1.70 Å for the C3 and DNA molecules, respectively. As shown in Table 7, the average RMSD values of the simulated atomic positions from those in the X-ray crystal structures for the studied nucleases B1, C3, and D1, are only 0.22, 0.17, and 0.50 Å, respectively. Both the MD-simulated structures and the energy-minimized structures are all consistent with the corresponding X-ray crystal structures.

Table 7 Average RMSD values (Å) of the calculated atomic positions of nucleases from those in the X-ray crystal structures

Complex	MM	MD
B1	0.12	0.22
C3	0.16	0.17
D1	0.27	0.50

Visual analysis of the C3 structure obtained from the trajectory in the corresponding simulation supports the observation data described above, as shown in Figs. 4 and 5. It can be seen from Fig. 4 that the nuclease C3 is stable in the minor groove of DNA during the course of the simulation. Figure 5, which shows one typical snapshot of the simulations for the C3 nuclease bound to DNA, exhibits the orientation of the C3 nuclease bound to DNA and the distance between central Cu ion of C3 nuclease and C4′H atom of the sugar in the DNA. The average distance between central copper ion and C4′H atom of nearest sugar is about 3.5 Å (shown in Fig. 6). Based on the cleavage mechanism [89], the obtained distances between copper center and C4′H atom is appropriate for inserting an substrate, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) or hydroxyl radical (·OH), which can abstract the C4′H atom from the DNA deoxyribose sugar to occur a redox cleavage procedure. The current simulated results support the proposed cleavage mechanisms of metallonucleases to DNA reported by some experimental and theoretical studies [14, 89, 90]. As shown in Fig. 7, the MD-simulated structure of C3 nuclease (after 10 ns MD simulation) can overlap with the X-ray crystal structure

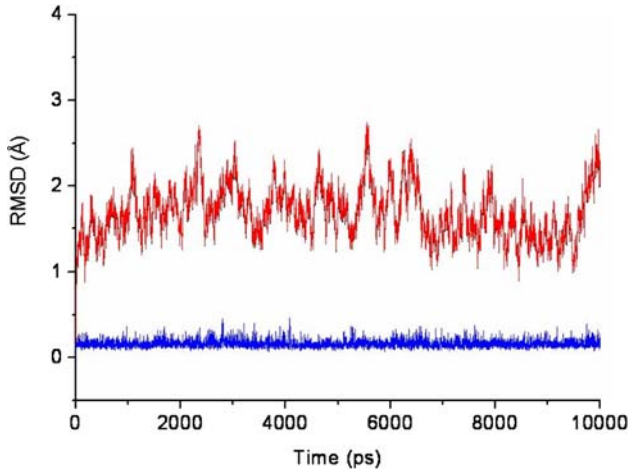

Fig. 4 RMSD values with respect to the starting structure in the simulation of DNA + C3 (*black*), DNA (*red*) and C3 nuclease (*blue*)

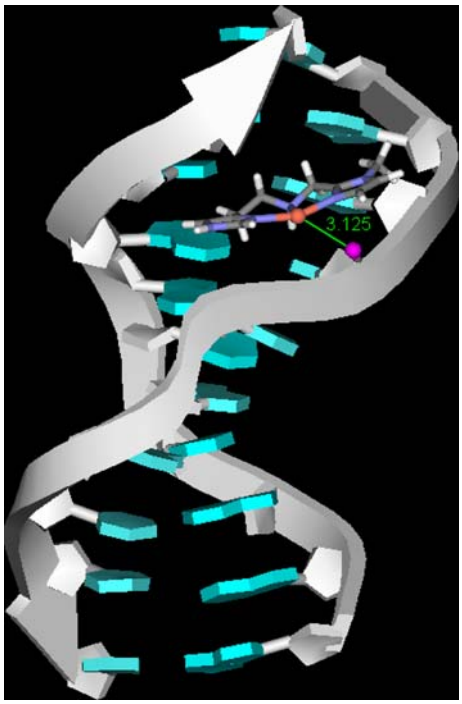

Fig. 5 Snapshot of structure of C3 nuclease (*tube*) bound to DNA with the distance (Å) between copper center (*orange*) and C4′H (*rose*) of the sugar

very well. All results predict that the AMBER force field parameters around the copper center evaluated by this work for the studied copper nucleases and the calculated RESP charges are reasonable for the simulations of the DNA-combining systems. In summary, the preparation of the force field parameters and atomic charges for the four typical classes of copper-based nucleases is an important task for the MD simulations of biological systems involving copper-based nucleases.

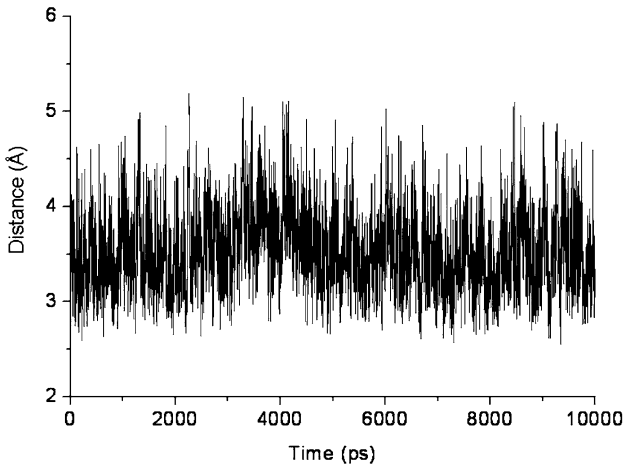

Fig. 6 Distances between copper center and C′4H in the sugar of DNA for the C3 nuclease–DNA complex during the course of simulation

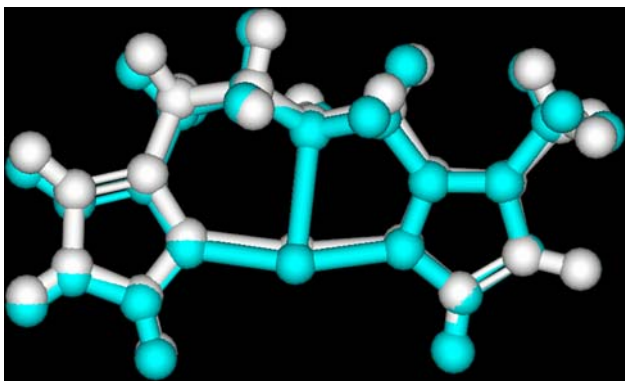

Fig. 7 Superposition of the optimized structure (*blue*) and snapshot after MD simulations (*white*) for nuclease C3

4 Conclusions

Reasonable AMBER force field parameters for 12 copper-based artificial nucleases have been evaluated by using the three-point approach based on first-principle quantum chemical calculations. The application of the three-point approach for evaluating force field parameters involves only the calculation of the secondary derivative of molecular energy with respect to a bond length or bond angle via the energies of three geometries at adjacent bond lengths or bond angles. The practical application of this approach is reliable and very computationally efficient. The protocol of RESP atomic charges has been used to calculate the atomic charge distributions of the studied copper nucleases. The determined force field parameters and RESP atomic charges have been validated by performing practical MM calculations and MD simulations on some representative nucleases. The RMSD analysis of MM-energy minimized and MD-simulated structures in

comparison with the corresponding X-ray crystal structures for three studied nucleases, i.e. B1, C3, and D1, suggests that the developed force field parameters and atomic charges can consistently reproduce the molecular geometries and conformations of the X-ray structures, including the reasonable prediction of the rigid features of these nuclease structures. At the same time, MD simulations on three nucleases binding to DNA were carried out to validate the efficiency of the calculated force field parameters and atomic charges in this work. The simulation results demonstrated that each docked nuclease remains stable in the minor groove of DNA during the MD simulations and that the cleavage of the DNA-strand by the copper nucleases characterizes the spatial accessibility and orientation of the sugar H's whose abstraction is a required precondition, which has been proposed previously in some experimental studies [3]. All of the computational results suggest that RESP charges and the new AMBER force field parameters around copper center evaluated in this work are reliable and suitable for MD simulations on the nuclease–DNA binding systems. These force field parameters are expected to be valuable for computational studies of copper-based nucleases associated with biological macromolecules.

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