Development and Validation of Empirical Force Field Parameters for Netropsin

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The netropsin molecule preferentially binds to the four consecutive A·T base pairs of the DNA minor groove and could therefore inhibit the expression of specific genes. The understanding of its binding on a molecular level is indispensable for computer-aided design of new antitumor agents. This knowledge could be obtained via molecular dynamics (MD) and docking simulations, but in this case appropriate force field parameters for the netropsin molecule should be explicitly defined. Our parametrization was based on the results of quantum chemical calculations. The resulting set of parameters was able to reproduce bond lengths, bond angles, torsional angles of the ab initio minimized geometry within 0.03 Å, 3 deg and 5 deg, respectively, and its vibrational frequencies with a relative error of 4.3% for low and 2.8% for high energy modes. To show the accuaracy of the developed parameters we calculated an IR spectrum of the netropsin molecule using MD simulation and found it to be in good agreement with the experimental one. Finally, we performed a 10 ns long MD simulation of the netropsin−DNA complex immersed in explicit water. The overall complex conformation remained stable at all times, and its secondary structure was well retained.

1. INTRODUCTION

The interactions of small molecules bound to B-DNA have been intensively studied. Drugs interact with DNA either by intercalation, by binding to the minor or major groove, through cross-linking, or by combinations of these interactions. Structural data indicate that in most cases the small drug molecules act as rigid bodies with little conformational change. In contrast to intercalation groove binding does not induce major alteration of the DNA structure. The groove binders frequently target specific DNA sequences and can therefore inhibit transcription and expression of specific genes. Thus sequence-specific binding drugs are of great interest as antitumor agents.[1]

The majority of small molecules binds to the minor groove of B-DNA. One of the best studied minor groove binders is dicationic netropsin (Figure 1), a natural oligopeptide antibiotic that has been isolated from cultures of *Streptomyces netropsis*.[2] The molecule is bowed with the amide groups on the concave side and the carbonyl and methyl groups on the convex side. Netropsin molecules are able to penetrate deep into the minor groove of DNA where they preferentially bind to the four consecutive A·T base pairs always in a 1:1 high-affinity mode.[3−5] This sequence specificity can be explained by the better electrostatic interaction of the positively charged ligand with the highly negative electrostatic potential of B-DNA in the minor groove of A·T base pairs, by the greater propeller twist of A·T regions resulting in a narrower minor groove and thus better van der Waals contacts, and by the concave shape of netropsin, which fits exactly in the convex minor groove of DNA and is only disturbed by the exocyclic amino N2 group of guanine. In addition, the drug can form hydrogen bonds to O2 of thymine and N3 of adenine. Evidently, electrostatic repulsion between the charged ends of netropsin molecules prevents their side by side arrangements in the minor groove of DNA. Binding to single stranded nucleic acids is negligible.

Studies of structural and dynamical aspects of netropsin−DNA complexes' behavior are important for understanding the intimate molecular mechanisms of their functioning. Moreover, knowledge of ligand binding on a molecular level is indispensable for rationalization of experimental data and will help in computer-aided design of new antitumor drugs controlling transcription. Such information may be obtained via molecular dynamics (MD) and docking simulations, but in this case appropriate force field parameters for the netropsin molecule should be explicitly defined. According to our knowledge, no attempts to parametrize netropsin for molecular simulations with force fields have been reported earlier. In the present study we describe development of the CHARMM force field parameters for the netropsin molecule, as empirical force field parameters for DNA already exist in the CHARMM parameter and topology files.[6] The main objective of this study is to obtain a correct and efficient molecular force field to perform MD and docking simulations of the netropsin molecule. Because the experimental data for such systems are scarce, development of the force field was done based on the results of ab initio quantum chemical calculations. Deriving molecular mechanics (MM) force fields from ab initio data is a common and powerful approach that has been successfully applied to numerous classes of molecules—from coordination complexes to peptides and proteins.[7,8]

To check the vibrational properties a MD simulation of the netropsin molecule in vacuo was performed, and its

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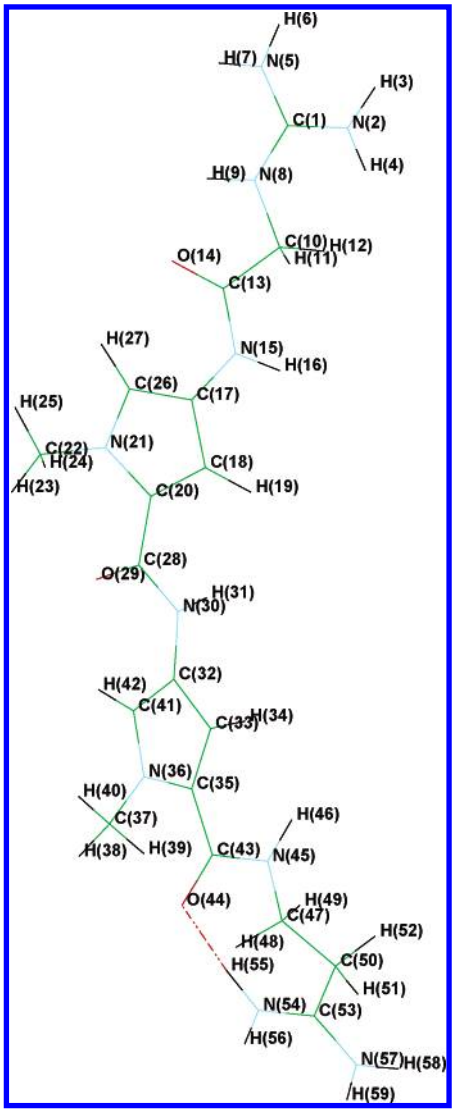

Figure 1. The atomic numbering scheme of netropsin used in the parametrization. The molecule is placed in its optimal geometry.

vibrational spectrum was calculated. The results were compared with the experimental IR spectrum of netropsin dihydrochloride in the crystal phase. To test the ability of the developed force field parameters to study biologically relevant problems, a MD simulation of netropsin bound to the minor groove of 12 base pairs long segment of DNA, surrounded by explicit water and potassium ions, was performed. A MD-based analysis of stability of the overall spatial structure of the netropsin−DNA complex represents an important criterion concerning applicability of the proposed force field parameters to structural and dynamical studies of the netropsin molecule.

2. METHODS

All calculations were carried out on the CROW clusters at the National Institute of Chemistry in Ljubljana.[9−11] The experimental IR spectrum of netropsin dihydrochloride in the crystal phase was recorded on a Perkin-Elmer Spectrum BX FTIR spectrophotometer.

2.1. Ab Initio and MM Calculations. The CHARMM potential energy function was taken in the form

$$E_{\text{pot}} = \sum K_{\text{b}}(b - b_0)^2 + \sum K_\theta(\theta - \theta_0)^2 + \sum K_\chi[1 + \cos(n\chi - \sigma)] + \sum\left(\epsilon_{ij}\left[\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{\epsilon_D r_{ij}}\right) \quad (1)$$

where K_{b} and K_θ are the stretching and bending force constants; b_0 and θ_0 represent the equilibrium bond length and bond angle; K_χ is the torsional energy barrier for the Fourier term with periodicity n and phase σ for dihedral angle χ; q_i and q_j represent fractional charges localized at the nuclei; ϵ_{ij} and $R_{\min,ij}$ are the van der Waals parameters; r_{ij} represents the distance between atoms i and j.

All ab initio calculations were performed in vacuo using GAUSSIAN03 software.[12] Visualization of the systems was done using the MOLDEN program.[13] The biologically active conformation of the netropsin molecule, which could be found in the Brookhaven Protein Data Bank (entry 101D), served as a starting geometry.[14−16] Geometry optimization of the drug and calculation of vibrational frequencies of its normal modes were performed at the B3LYP/6-31G(d) level of theory. All calculated vibrational frequencies were real, indicating that the geometry minimization was correctly performed. The optimized geometry of netropsin, with a root-mean-square difference (RMSD) of the gradient equal to 0.000003 au, was used to determine the equilibrium geometric parameters.

Partial atomic charges employed in subsequent calculations were derived using the standard CHARMM procedure.[7] All the aliphatic hydrogens were assigned a charge of 0.09, with the carbon charge adjusted to yield a zero charge. The charge of aromatic carbons and hydrogens was set to −0.115 and 0.115, respectively. At points of substitution of aromatic groups, the carbon charge was set to 0.0. Then we calculated minimum interaction energies and distances for individual water molecules interacting with remaining sites on the drug molecule at the HF/6-31G(d) level of theory. The ab initio distances of hydrogen bonds were decreased by 0.1 Å to yield the target data. Finally, partial atomic charges were iteratively optimized by hand until satisfactory agreement between force field based results and target data was reached. Mulliken HF/6-31G(d) charges served as a starting point. TIP3-water was employed in empirical calculations. During the whole procedure we kept charges of both hydrogens and nitrogens in the amino groups N2, N5, N54, and N57 the same. An additional set of fractional atomic charges was derived using the standard AMBER practice.[7] The RESP charge-fitting procedure was applied to reproduce HF/6-31G(d) electrostatic potential (ESP) around the netropsin molecule.[17,18] Charges of all hydrogens forming the same methyl or methylen group were restricted to the same value. The charges of both hydrogens and nitrogens in the amino groups N2, N5, N54, and N57 were also restricted to the same value. Standard van der Waals parameters from CHARMM22 protein topology and parameter files were applied as they are to a large extent transferable between similar molecules.[7]

Initial stretching, bending, and torsion force constants for netropsin were taken from the standard CHARMM22 library.

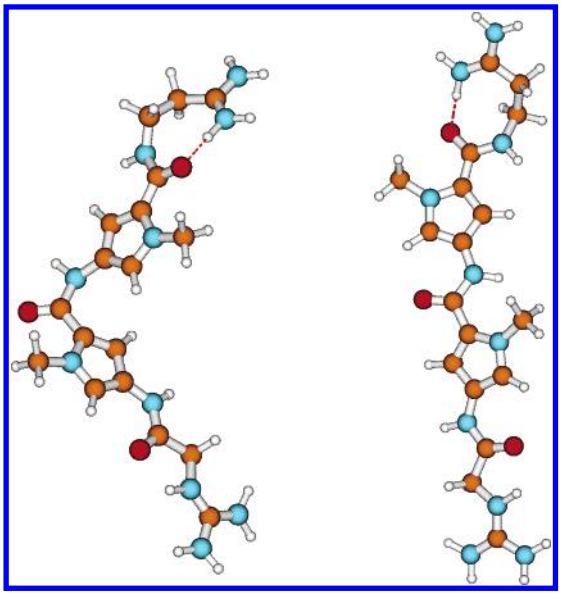

Figure 2. Two characteristic conformations of the netropsin molecule.

As the drug molecule was too big we had to cut bonds N8−C10, N15−C17, C20−C28, N30−C32, C35−C43, and C47−C50 to obtain six smaller model compounds. First, appropriate equilibrium geometric parameters, partial atomic charges, and van der Waals parameters were derived for each of them using procedures already described above. Then for every model compound the force constants were iteratively varied by hand until satisfactory agreement between vibrational frequencies of the same normal modes calculated via ab initio and empirical methods was reached. Finally, all six compounds had to be reconnected, and missing force constants had to be devised using the same iterative procedure.

Two additional conformations of netropsin were obtained with a conformational analysis of the drug molecule in vacuo employing the semiempirical PM3 method.[19] We performed subsequent energy minimization at the B3LYP/6-31G(d) level of theory. The first and the second conformation are presented in Figure 2. Using the same two starting structures we also carried out geometry optimization employing empirical methods.

All the force field based calculations were carried out using the CHARMM program. Energy minimization was performed with the Adoptive Base Newton−Raphson algorithm until RMSD of the gradient became lower than 0.00001 kcal (mol Å)$^{-1}$. Vibrational analysis was carried out on the fully minimized structures. Visualization of the systems was done via VMD software.[20]

2.2. MD Calculations. A MD simulation of a single netropsin molecule was carried out. The starting structure was obtained from Brookhaven Protein Data Bank (entry 101D). Initially, the energy minimization of netropsin was performed. Then the system was subjected to 100 ps of temperature equilibration at 300 K, followed by 150 ps of unrestrained MD collection run using the NVE ensemble. All the nonbonded interactions were accounted for in the forces calculations. Finally, an IR spectrum of the netropsin molecule was calculated from the last 50 ps of MD trajectory with the help of equation[21−25]

$$I(\omega) \propto \int_0^{\infty} \left\langle \frac{d\mu(t)}{dt} \cdot \frac{d\mu(0)}{dt} \right\rangle \cos(\omega t) dt \qquad (2)$$

The second MD simulation was performed on the Dickerson-Drew sequence d(CGCGAATTCGCG) from the netropsin−DNA complex. Atomic coordinates were taken from Brookhaven Protein Data Bank (entry 101D). The complex was placed in a cubic box filled with 7057 TIP3-waters and 22 ions of potassium. Periodic boundary conditions were applied. The edge of the cube was 5.95 nm long. Initially, the steepest descent geometry optimization of water molecules was carried out, followed by 50 ps of MD simulation of TIP3-waters using the NPT ensemble. Then the whole system was subjected to 50 ps of temperature equilibration at 300 K. A subsequent 10 ns unrestrained MD collection run was performed at normal pressure in the NPT ensemble. Long-range electrostatic interactions were treated in the framework of the PME summation. Nonbonded interactions were truncated using the spherical cutoff 1.8 nm. Finally, RMSD values of atom positions in the complex relative to their initial positions in the X-ray structure were calculated from the MD trajectory.

3. RESULTS AND DISCUSSION

3.1. Results of ab Initio and MM Calculations. The applied names of atoms forming the netropsin molecule are shown in Figure 1. The molecule is placed in its optimal geometry. Adequate atomic types and partial atomic charges are shown in Table 1. Suitable van der Waals parameters are presented in Table 2. All stretching parameters are placed in Table 3. Adequate bending parameters can be found in Table 4. Finally, suitable torsional parameters are shown in Table 5.

The developed force field parameters were used to perform energy minimization. Starting geometry of the netropsin molecule was taken from the Brookhaven Protein Data Bank (entry 101D). Bond lengths, bond angles, and torsional angles were all within 0.03 Å, 3 deg and 5 deg, respectively, off the ab initio calculated values. Corresponding internal coordinate (IC) tables could be found in the Supporting Information. We report all the bond distances, bond angles, and torsional angle values that are needed to reconstruct the Cartesian coordinates of the molecule. This results point to the ability of the new force field parameters to reproduce well geometric features of the netropsin molecule.

Optimized partial atomic charges were able to reproduce all ab initio calculated minimum interaction energies of netropsin−water supramolecular complexes within 0.1 kcal mol^{-1}. Hydrogen bond lengths were on average −0.08 Å off their target values. Results in terms of individual interactions are presented in the Supporting Information. Developed force field parameters therefore yield satisfactory condensed phase properties.

The vibrational analysis was performed on the optimized geometry of netropsin using ab initio and force field based methods. The average relative difference between the calculated frequencies of the same normal modes was 4.3% for the low (under 1500 cm^{-1}) and 2.8% for the high energy region. This reveals a rather good performance of the developed force field parameters to reproduce vibrational properties of the netropsin molecule. Usually, the best

Table 1. Atom Types and Partial Atomic Charges in Electron Charge Units for the Netropsin Molecule

name	type	CHARMM q	RESP q	name	type	CHARMM q	RESP q
C1	2C	0.990	0.802	H31	6H	0.413	0.329
N2	2NC2	−0.720	−0.905	C32	7CY	0.000	0.152
H3	2HC	0.420	0.460	C33	7CY	−0.115	−0.410
H4	2HC	0.420	0.460	H34	7HA	0.115	0.231
N5	2NC2	−0.720	−0.905	C35	7CA	0.000	−0.068
H6	2HC	0.420	0.460	N36	7NY	0.222	0.036
H7	2HC	0.420	0.460	C37	7CT3	−0.270	−0.132
N8	2NC2	−0.526	−0.362	H38	7HA	0.090	0.097
H9	2HC	0.341	0.287	H39	7HA	0.090	0.097
C10	4CT2	−0.180	−0.058	H40	7HA	0.090	0.097
H11	4HA	0.090	0.086	C41	7CA	−0.115	−0.144
H12	4HA	0.090	0.086	H42	7HA	0.115	0.202
C13	4CC	0.704	0.547	C43	5C	0.775	0.596
O14	4O	−0.516	−0.489	O44	5O	−0.655	−0.551
N15	4NH2	−0.958	−0.396	N45	5NH1	−0.734	−0.545
H16	4H	0.451	0.333	H46	5H	0.328	0.328
C17	7CY	0.000	0.078	C47	5CT2	−0.180	0.111
C18	7CY	−0.115	−0.370	H48	5HA	0.090	0.081
H19	7HA	0.115	0.200	H49	5HA	0.090	0.081
C20	7CA	0.000	−0.107	C50	5CT3	−0.180	−0.133
N21	7NY	0.215	0.063	H51	5HA	0.090	0.091
C22	7CT3	−0.270	−0.146	H52	5HA	0.090	0.091
H23	7HA	0.090	0.102	C53	3C	0.950	0.593
H24	7HA	0.090	0.102	N54	3NC2	−0.689	−0.748
H25	7HA	0.090	0.102	H55	3HC	0.552	0.434
C26	7CA	−0.115	−0.153	H56	3HC	0.443	0.416
H27	7HA	0.115	0.184	N57	3NC2	−0.689	−0.748
C28	6C	0.732	0.670	H58	3HC	0.443	0.416
O29	6O	−0.530	−0.521	H59	3HC	0.443	0.416
N30	6NH1	−0.945	−0.486				

Table 2. van der Waals Parameters for the Netropsin Molecule[a]

type	ϵ_i	$R_{\min,i}$	type	ϵ_i	$R_{\min,i}$
2HC	−0.046	0.2245	6H	−0.046	0.2245
2C	−0.110	2.0000	6C	−0.110	2.0000
2NC2	−0.200	1.8500	6NH1	−0.200	1.8500
4H	−0.046	0.2245	6O	−0.120	1.7000
4CC	−0.070	2.0000	5H	−0.046	0.2245
4O	−0.120	1.7000	5C	−0.110	2.0000
4NH2	−0.200	1.8500	5NH1	−0.200	1.8500
4CT2	−0.055	2.1750	5O	−0.120	1.7000
4HA	−0.022	1.3200	5CT2	−0.055	2.1750
7HA	−0.022	1.3200	5CT3	−0.055	2.1750
7CA	−0.070	1.9924	5HA	−0.022	1.3200
7NY	−0.200	1.8500	3HC	−0.046	0.2245
7CY	−0.070	1.9924	3C	−0.110	2.0000
7CT3	−0.080	2.0600	3NC2	−0.200	1.8500

[a] The well depths (ϵ_i) in kcal mol^{-1} and $R_{\min,i}$ in Å are presented.

correspondence between B3LYP/6-31G(d) and experimental frequencies is achieved if the first are scaled by a factor of 0.96.[26] Therefore we scaled the force constants by a factor of 0.92. To obtain a good agreement between the experimental and calculated IR spectrum of netropsin we had to scale the N−H stretching force constants by a factor of 0.81 and C−O and C−N stretching force constants by a factor of 0.77. The scaled force constants are presented in Table 3. They were employed in all empirical calculations.

The two conformations of netropsin depicted in Figure 2 were retained via force field based geometry optimizations with RMSD values calculated over all atoms 0.91 and 1.67 Å, respectively. The energy difference between the first and the second conformation calculated using GAUSSIAN03 was −7.7 kcal mol^{-1} and −7.6 kcal mol^{-1} by empirical methods. The energy difference between the optimal geometry and

Table 3. Bond Parameters for the Netropsin Molecule[a]

type	type	K_b	b_0	type	type	K_b	b_0
2NC2	2HC	413.	1.01	7CA	6C	328.	1.48
2NC2	2C	421.	1.34	6NH1	6H	401.	1.01
2NC2	4CT2	263.	1.45	6NH1	6C	333.	1.38
4HA	4CT2	331.	1.10	6O	6C	386.	1.23
4CC	4CT2	229.	1.56	6NH1	7CY	340.	1.40
4O	4CC	411.	1.23	5C	7CA	328.	1.45
4NH2	4CC	331.	1.35	5CT3	5HA	340.	1.10
4NH2	4H	411.	1.01	5CT3	5CT2	234.	1.55
4NH2	7CY	340.	1.41	5CT2	5HA	332.	1.09
7CY	7CY	373.	1.41	5CT2	5NH1	355.	1.46
7CY	7HA	372.	1.08	5NH1	5H	397.	1.01
7CY	7CA	360.	1.39	5NH1	5C	347.	1.37
7CA	7HA	377.	1.08	5C	5O	410.	1.25
7CA	7NY	295.	1.36	3NC2	3HC	407.	1.02
7NY	7CT3	267.	1.47	3NC2	3C	386.	1.31
7CT3	7HA	341.	1.09	5CT3	3C	333.	1.52

[a] The stretching force constants in kcal mol^{-1} Å^{-2} and the equilibrium bond lengths in Å are presented.

Table 4. Angle Parameters for the Netropsin Molecule[a]

type	type	type	K_θ	θ_0	type	type	type	K_θ	θ_0
2HC	2NC2	2HC	27.4	117.0	7CA	6C	6O	72.0	122.5
2C	2NC2	2HC	43.2	121.4	7CA	6C	6NH1	112.	115.4
2NC2	2C	2NC2	36.0	129.2	7CY	6NH1	6H	37.8	116.8
2HC	2NC2	4CT2	42.2	110.8	6H	6NH1	6C	36.9	118.1
2NC2	4CT2	4HA	53.9	111.0	7CY	6NH1	6C	85.0	124.4
2NC2	4CT2	4CC	45.0	104.7	6NH1	6C	6O	99.0	122.0
4HA	4CT2	4HA	36.8	108.9	6NH1	7CY	7CY	55.3	125.6
4CT2	4CC	4O	27.0	118.9	6NH1	7CY	7CA	45.0	127.5
4CT2	4CC	4NH2	36.0	115.8	5C	7CA	7NY	45.0	122.5
4O	4CC	4NH2	99.0	125.3	5C	7CA	7CY	45.0	130.0
4CC	4NH2	4H	52.1	118.4	5O	5C	7CA	72.0	122.8
4HA	4CT2	4CC	40.5	109.8	5NH1	5C	7CA	112.	117.4
2C	2NC2	4CT2	56.0	127.5	5HA	5CT3	5HA	38.6	106.4
4CC	4NH2	7CY	85.5	125.5	5HA	5CT3	5CT2	35.9	108.2
4H	4NH2	7CY	37.8	116.0	5CT3	5CT2	5HA	35.9	106.6
4NH2	7CY	7CY	45.0	124.8	5CT3	5CT2	5NH1	89.9	115.8
4NH2	7CY	7CA	45.0	127.5	5HA	5CT2	5NH1	53.9	107.7
7CY	7CY	7HA	55.3	126.6	5HA	5CT2	5HA	38.6	107.0
7CY	7CY	7CA	54.0	107.0	5CT2	5NH1	5H	44.9	117.3
7CY	7CA	7HA	29.2	130.1	5CT2	5NH1	5C	49.5	122.5
7CY	7CA	7NY	139.	108.1	5H	5NH1	5C	36.0	117.8
7HA	7CY	7CA	19.8	126.4	5NH1	5C	5O	89.1	119.8
7HA	7CA	7NY	29.2	121.8	5CT2	5CT3	3C	46.8	118.1
7CA	7NY	7CA	54.0	109.4	3HC	3NC2	3HC	22.5	116.6
7CA	7NY	7CT3	108.	123.0	3C	5CT3	5HA	47.2	107.3
7NY	7CT3	7HA	46.3	107.7	3C	3NC2	3HC	44.1	120.9
7HA	7CT3	7HA	31.9	109.6	3NC2	3C	5CT3	56.0	116.8
7CY	7CA	6C	45.0	131.0	3NC2	3C	3NC2	82.8	121.7
7NY	7CA	6C	45.0	121.2					

[a] The bending force constants in kcal mol^{-1} rad^{-2} and the equilibrium bond angles in deg are presented.

the second conformation calculated via ab initio methods was −10.8 kcal mol^{-1} and −10.7 kcal mol^{-1} employing force field based methods. These results point to the ability of the developed force field parameters to reproduce well conformational characteristics of the netropsin molecule.

3.2. Results of MD Calculations. To check the vibrational properties of the netropsin molecule, its MD simulation was carried out. The system was well equilibrated after 100 ps of MD run. Analysis of the resulting MD trajectory shows stable behavior of the netropsin molecule: no significant distortions of its structure were observed. All bond lengths and bond angles fluctuated near their equilibrium values. Finally, the IR spectrum of the netropsin molecule was

Table 5. Dihedral Parameters for the Netropsin Molecule[a]

type	type	type	type	K_χ	n	δ	type	type	type	type	K_χ	n	δ
2NC2	2C	2NC2	2HC	1.9	2	163.6	6NH1	6C	7CA	7NY	1.3	2	−145.4
2NC2	2C	2NC2	4CT2	2.2	2	157.8	6NH1	6C	7CA	7CY	0.85	1	−160.1
2C	2NC2	4CT2	4HA	0.18	3	25.2	7CY	6NH1	6C	7CA	2.99	2	179.8
2C	2NC2	4CT2	4CC	0.18	1	8.7	6H	6NH1	6C	7CA	1.7	1	−170.0
2HC	2NC2	4CT2	4CC	0.16	1	−177.1	6H	6NH1	6C	6O	4.27	2	−160.8
2HC	2NC2	4CT2	4HA	0.08	3	−167.4	7CY	6NH1	6C	6O	4.27	2	179.0
2NC2	4CT2	4CC	4O	2.7	1	178.7	6C	6NH1	7CY	7CY	2.2	1	4.3
2NC2	4CT2	4CC	4NH2	2.7	1	−1.1	6C	6NH1	7CY	7CA	2.2	1	−176.3
4HA	4CT2	4CC	4O	0.08	3	175.5	6H	6NH1	7CY	7CY	2.2	1	174.3
4HA	4CT2	4CC	4NH2	0.08	3	3.9	6H	6NH1	7CY	7CA	2.2	1	−6.3
4CT2	4CC	4NH2	4H	2.6	1	−179.3	6NH1	7CY	7CY	7HA	6.7	1	−178.0
4O	4CC	4NH2	4H	2.6	1	−0.5	6NH1	7CY	7CY	7CA	6.7	1	−0.8
4O	4CC	4NH2	7CY	4.27	1	−179.1	6NH1	7CY	7CA	7HA	6.7	1	−179.7
4CT2	4CC	4NH2	7CY	2.99	1	0.6	6NH1	7CY	7CA	7NY	6.7	1	0.9
4CC	4NH2	7CY	7CY	2.2	1	−0.5	5C	7CA	7NY	7CT3	6.7	1	178.6
4CC	4NH2	7CY	7CA	2.2	1	−179.6	5C	7CA	7NY	7CA	6.7	1	−1.6
4H	4NH2	7CY	7CY	2.2	1	−179.1	5C	7CA	7CY	7CY	6.7	1	2.0
4H	4NH2	7CY	7CA	2.2	1	1.8	5C	7CA	7CY	7HA	6.7	1	179.2
4NH2	7CY	7CY	7HA	6.7	1	177.8	5O	5C	7CA	7NY	2.2	1	167.7
4NH2	7CY	7CY	7CA	6.7	1	1.0	5O	5C	7CA	7CY	2.2	1	−14.5
4NH2	7CY	7CA	7HA	6.7	1	178.7	5NH1	5C	7CA	7NY	2.2	1	−12.2
4NH2	7CY	7CA	7NY	6.7	1	−1.2	5NH1	5C	7CA	7CY	2.2	1	165.6
7HA	7CY	7CY	7CA	7.6	1	−3.0	5H	5NH1	5C	7CA	4.5	1	178.0
7HA	7CY	7CA	7NY	7.9	1	3.2	5CT2	5NH1	5C	7CA	2.99	1	−20.3
7CY	7CY	7CA	7HA	6.7	1	−0.5	5HA	5CT3	5CT2	5NH1	0.18	3	18.0
7CY	7CY	7CA	7NY	5.8	1	180.0	5HA	5CT3	5CT2	5HA	0.09	3	−63.0
7CY	7CA	7NY	7CA	6.3	1	179.7	5CT3	5CT2	5NH1	5H	0.18	1	85.3
7CY	7CA	7NY	7CT3	6.3	1	0.1	5CT3	5CT2	5NH1	5C	0.18	1	−76.5
7HA	7CA	7NY	7CT3	6.7	1	−179.9	5HA	5CT2	5NH1	5H	1.7	3	−106.8
7HA	7CA	7NY	7CA	6.7	1	0.5	5HA	5CT2	5NH1	5C	0.09	3	127.8
7CA	7CY	7CY	7CA	4.9	1	−179.7	5CT2	5NH1	5C	5O	1.8	1	159.8
7CA	7NY	7CT3	7HA	0.27	6	136.8	5H	5NH1	5C	5O	6.7	1	−2.0
6C	7CA	7NY	7CT3	6.7	1	−177.7	5NH1	5CT2	5CT3	3C	0.18	1	95.1
6C	7CA	7NY	7CA	6.7	1	1.9	5HA	5CT2	5CT3	3C	0.17	3	−74.1
6C	7CA	7CY	7CY	6.7	1	−2.4	5CT2	5CT3	3C	3NC2	0.19	2	−147.2
6C	7CA	7CY	7HA	6.7	1	−179.2	5HA	5CT3	3C	3NC2	0.005	6	−69.6
6O	6C	7CA	7NY	1.3	2	−144.8	3NC2	3C	3NC2	3HC	2.8	2	−179.6
6O	6C	7CA	7CY	1.3	2	−139.4	5CT3	3C	3NC2	3HC	2.8	2	−177.7

[a] The torsional force constants in kcal mol^{-1}, periodicities and phases in deg are presented.

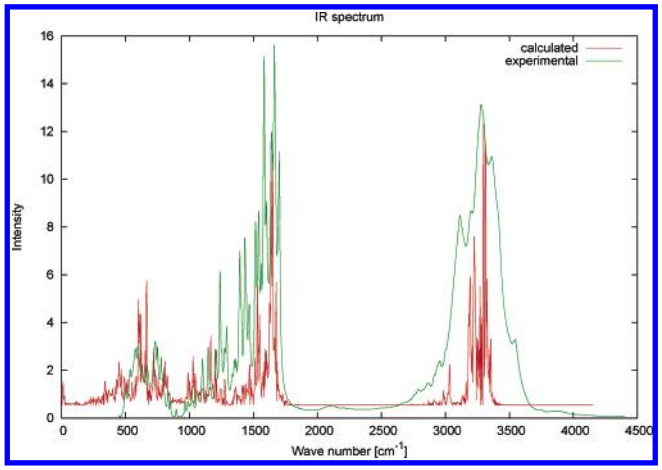

Figure 3. Comparison of the calculated IR spectrum of netropsin (red) with the experimental one (green).

calculated and compared to an experimental vibrational spectrum of crystalline netropsin dihydrochloride (Figure 3). The two spectra are in good agreement with one another.

Developed force field parameters should be consistent with other CHARMM force field parameters to provide adequate interactions between netropsin and various constituents of the system (for example, neighboring base pairs, waters, ions, and so forth). It is well-known that solvation effects play a significant role determining spatial structure and dynamics of molecules. Therefore, it is interesting to check the force field parameters in a short simulation with an explicit solvent. With this aim in view, 10 ns of MD of the netropsin−DNA complex in a box of explicit water was performed. The system was well equilibrated after 100 ps of MD run. The average RMSD value between starting (X-ray) and equilibrated MD structures was 2.51 Å (Figure 4). The overall complex conformation remained stable during dynamics in the explicit solvent. The secondary structure of the complex was well retained during the simulation. This provides a strong support to the proposed parametrization of the force field for netropsin—its interactions (including numerous H-bonds) with DNA and solvent environments did not disrupt the spatial structure of the complex. During the MD run, the conformation of the netropsin molecule was very close to that in the experimental and ab initio structures (Figure 4). The RMSD values show that netropsin does not move much in the minor groove as some crystallographic studies suggested.

4. SUMMARY

To summarize, a complete set of CHARMM force field parameters for the netropsin molecule was developed based on ab initio quantum chemical calculations. With small

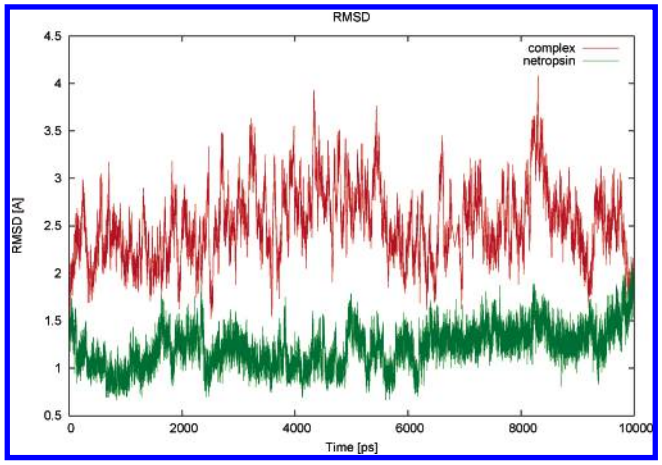

Figure 4. RMSD values (Å) of the whole complex with respect to the starting structure (red) and RMSD values of the ligand with respect to its starting structure (green).

modifications and proper scaling these parameters may be included into other popular force fields (AMBER, GAFF,[27,28] GROMOS, and so forth) widely used in molecular modeling of biologically relevant systems. The reliability of these new force field parameters was proved by molecular mechanics and MD simulations. The developed force field was able to reproduce well geometrical, vibrational, condensed phase, and conformational features of the netropsin molecule. The calculated IR spectrum was in good agreement with the experimental one. Short MD simulation of the netropsin−DNA complex in explicit water demonstrated a high degree of stability of its overall spatial structure. This provides strong support in favor of applicability of the proposed force field to structural and dynamical studies of biologically relevant systems. Finally, our simulation shows that netropsin does not move much in the minor groove as some crystallographic studies suggested. Such knowledge is essential for systematic modeling of new antitumor drugs controlling transcription via binding to a specific sequence of DNA.

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Supporting Information Available: All internal coordinates of the fully optimized structure of netropsin as well as all individual interaction energies and geometries of netropsin−water supramolecular complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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