CHAPTER VI

MOLECULAR DYNAMIC SIMULATION OF RHODAMIN-DNA COMPLEXES

Stacking interactions between DNA bases and a chromophore have been the subject of wildly studies, experimental as well as computational. [146] Information about structural features of chromophore-DNA complexes is of value and interest for a general understanding of specific recognition and binding by probing dye-DNA interactions, but also for a variety of specific problems ranging from the elucidation of properties of drug-DNA complexes to studies of charge transfer mediated by DNA. In the latter case, the chromophore plays the role of a charge injector. The efficiency of charge injection considerably depends on the donor and acceptor electronic coupling which in turn is determined by the relative position of neighboring π-systems. [147] Thus, for a detailed interpretation of spectroscopic data on the kinetics of electron-transfer in DNA, one needs to know the structural data of chromophore-DNA complexes.

Very useful structural information can be derived from NMR spectra. In fact, NMR spectroscopy is the primary source for structures of DNA in solution. A number of such studies have been reported. [148]

Complementary structure data can be obtained from molecular dynamics simulations. Organic chromophores that interact with DNA are polarizable, are π-electron systems. Therefore, dispersion interaction plays a key role by the formation of such complexes. It has been demonstrated that the stability of stacked complexes in the gas phase is dominated by the dispersion energy, whereas orientational preferences are determined by electrostatic forces. [149] Furthermore, hydrophobic forces can considerably affect the chromophore-DNA interaction in condensed phase. Thus, a method which treats dispersion, short-range as well as long-range electrostatic interactions should be applied when simulating such systems. Structure and stability of stacked aromatic systems can be well described by force fields which combine a simple electrostatic model and empirical potentials for the non-covalent molecular forces. In a comparison of several empirical potentials, the molecular force field

suggested by Cornell *et al.* [150] was found to provide a good description of the stacking interaction in π stacks. [151]

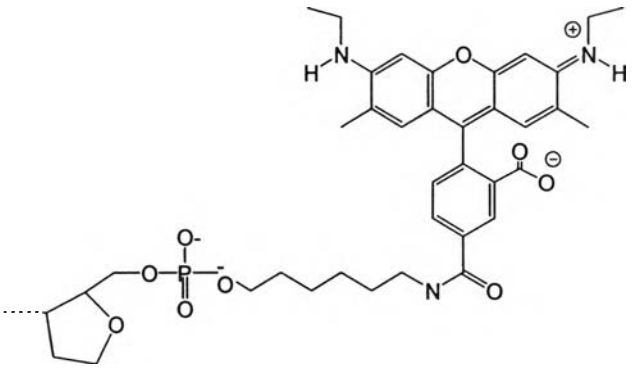

Figure 6.1 Chemical structure of the derivative rhodamine 6G (Rho).

Rhodamin 6G and its derivatives are very efficient fluorescent probes because of their photophysical properties and, therefore, have a considerable potential for spectroscopic study of charge transfer phenomena within DNA. In the present work MD simulations were performed to systematic study of structural and conformational features and dynamics of three complexes of rodamine-6G with DNA. The Rhodamine 6G derivative has been covalently connected to the defined sequence at phosphate atom through a linker chain.

As the main goal of this study is targeted to understand charge transfer in intercalated DNA complexes, in the present sections we investigate the molecular dynamic simulations of rhodamin 6G derivative (Rho) complexed with DNA in aqueous solution.

6.1 Models and Methods

The rhodamine 6G derivative studied in this work is shown on Figure 6.1. This is a neutral dipolar form (zwitter-ion), with the positive charge delocalized over a three-ring fragment and with a negatively charged carboxyl group. In the chromophore, three

flat conjugated cycles are responsible for spectroscopic properties; the carb-ethoxy-phenyl group is nearly perpendicular to the plane and may cause some steric constrained. The three investigated systems are where the Rho connects to DNA in the following three manners:

Rho–p-5'-CAAAGCG-3' 3'-GTTTCGC-5'	Rho–p-5'-GAAAGCG-3' 3'-CTTTCGC-5'	Rho–p-5'-GTTTCGC-3' 3'-CAAAGCG-5'
Rho-5C	**Rho-5G**	**Rho-5G'**

These three systems are very similar. In the first system, rhodamine is connected to 5'-Cytocine (5'-C) of the duplex (see Figure 6.2), whereas in the other two systems, the dye is bound to 5'-Gaunine (5'-G). The second system can be obtained from the first complex by "inversion" of the first CG pair and the last one is the inversion of the first system. For simplicity, the three simulated systems will be referred as Rho-5C, Rho-5G and Rho-5G', respectively

It should be noted that MD simulations of the complex of DNA with Rhodamine 6G derivative (Rho) cannot be performed routinely because the chromophore (see Figure 6.1) is not a standard residue and the corresponding force field parameters are not available in the AMBER residue libraries. Also atomic charges for this molecule have to be derived. Therefore, molecular structure of the Rho and linker atoms were fully optimized using B3LYP/6-31G* method in the Gaussian98 program. Then, a restrained electrostatic potential (RESP) charge, [152,153] which achieves a good performance in describing electrostatic filed, was applied. The electrostatic potential was calculated at the HF/6-31G* level to derive charges on the atoms. (detailed in Chapter5) This is same method used in the generation of AMBER force field. We have used a force field which is generated by Antechamber module in the AMBER package.

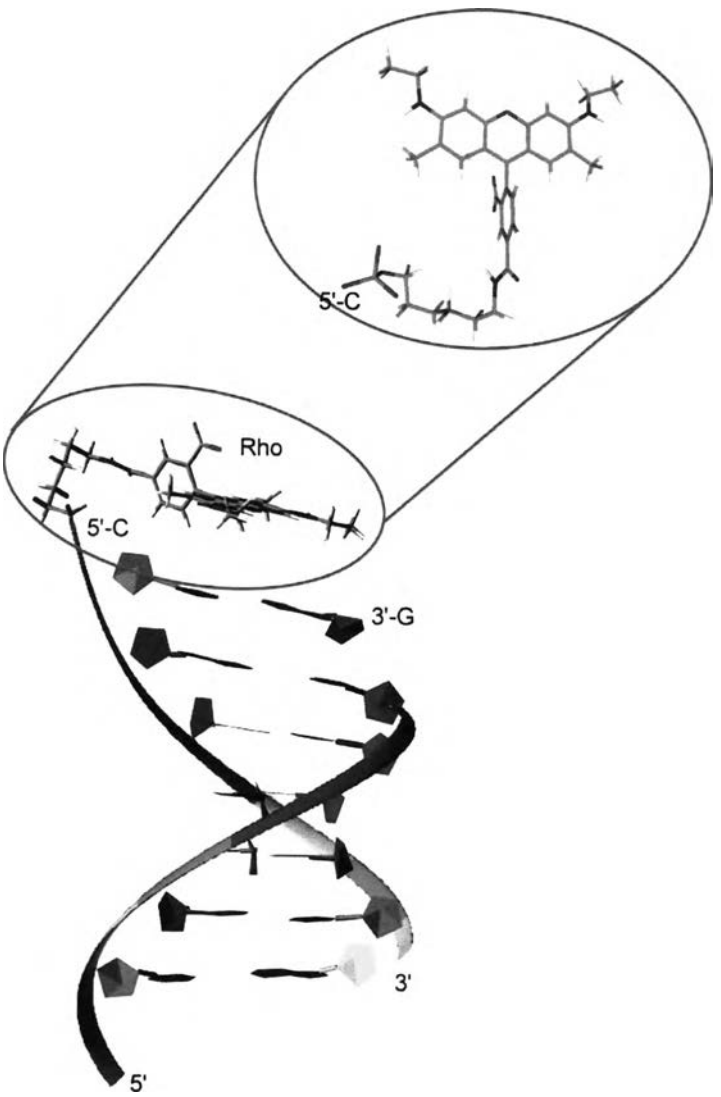

Figure 6.2 The model of the DNA duplex 5′-CAAAGCG-3′ with Rhodamine 6G derivative (Rho-5C)

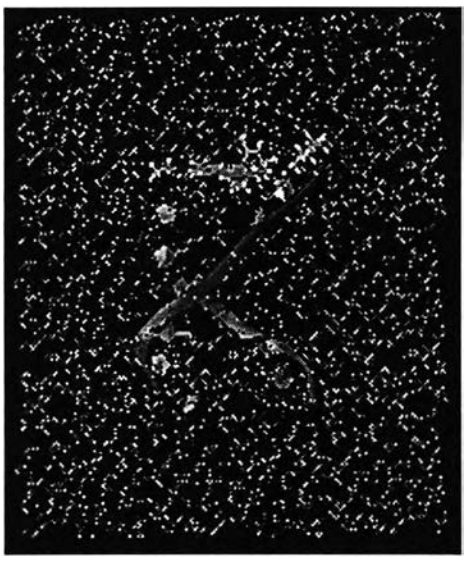

Figure 6.3 The initial set up for the simulation, Rho-5C and Na^+ ions with TIP3P water molecules

MD simulations were performed with the program AMBER 6. [154] The AMBER-95 force field described by Cornell *et al.* which comprises specifically improved parameters for the description of nucleic acids. [150] The simulations were carried out using module SANDER with SHAKE procedure on all hydrogen atoms. [155] A cut-off of 9 Å was applied for the Lennard-Jones interactions. 13 Na^+ were placed near the phosphates of the duplex to render the whole system neutral. The system was enclosed in a rectangular box which contained about 2900 TIP3P water molecules. [156] (see Figure 6.3) Periodic boundary conditions were applied using LEaP module. Simulations were studied up to 12 ns. with time step 1 fs.

We followed established procedures to construct the initial structures, obtain equilibration geometries, and generate their dynamics. [157,158] The simulations were performed at constant volume and total energy. The first step, Rho-DNA complexes were treated as rigid molecule with position restraint. We performed 1000 minimization step varying the positions of ions and water molecules. Then, the system was gradually heated to 300 K over 25 ps and was maintained at 300 K for 80 ps. MD production runs were performed using the particle mesh Ewald method [159] to account for long-range interactions. The total energy and volume of the simulations were monitored to make sure that they achieve constant values.

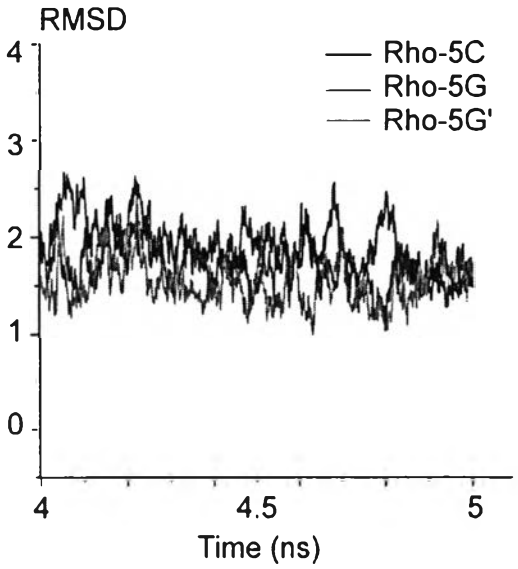

Figure 6.4 RMSD of DNA of Rho-5C, Rho-5G and Rho-5G' system with respect to the starting structure as a function of time for a 1 ns from 4-5 ns MD trajectory

The root mean square deviation (RMSD) of DNA in MD trajectory as a function of time shows in Figure 6.4. RMSD is computed from the mass-weighted mean square difference of all atoms of each snapshot. Using the starting structure as a reference, the averaged value is 1.9±0.2, 1.8±0.3 and 1.6±0.3 Å for Rho-5C, Rho-5G and Rho-5G', respectively. The result shows that the complex was stable along the simulation. This value agrees with the DNA duplex which studied by Cheatham and Kollman (2.90 Å).

6.2 Results and Discussion

6.2.1 Analysis of the chromophore position relative to the neighboring guanine

The relative position and orientation of Rho relative to the nearest guanine base can be described by six step-parameters: three translations (shift, slide and rise) and three rotational angles (tilt, roll and twist) shown in Figure 6.6. Slide and shift characterize relative movement of the fragments along the x and y axes, correspondingly. Rise is the distance between the fragment planes, i.e., the displacement along the *z*-axis. Tilt, roll, and twist parameters are defined as rotational angles about the *x*-, *y*- and *z*- axes, respectively. These parameters are very similar to

the base-step parameters used to specify the mutual positions of neighboring bases or base pairs in nucleic acids. [160]

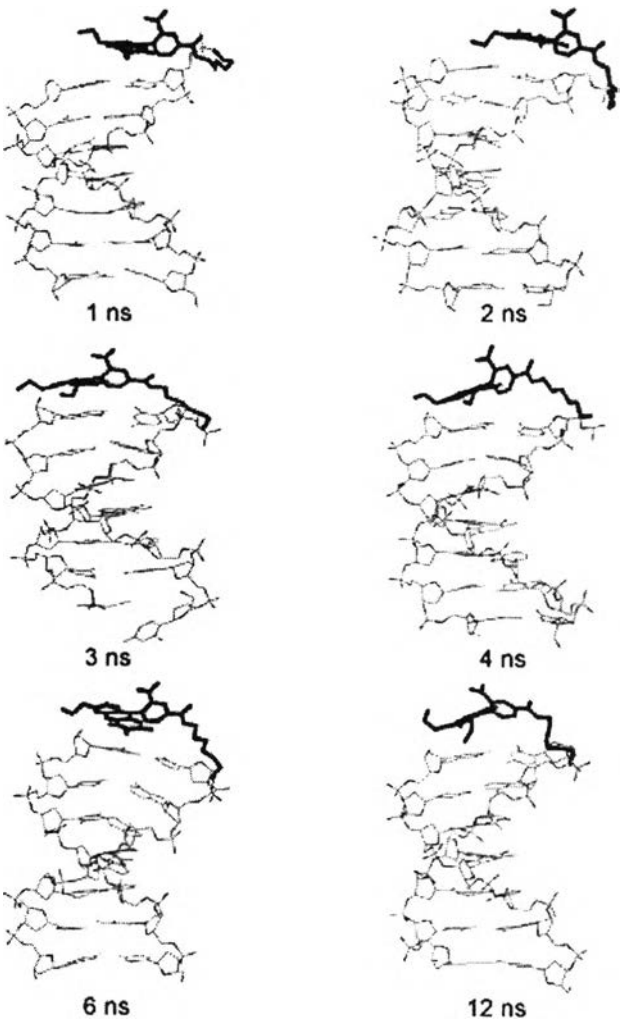

Figure 6.5 The six snapshots of Rho-5C complex taken from simulation.

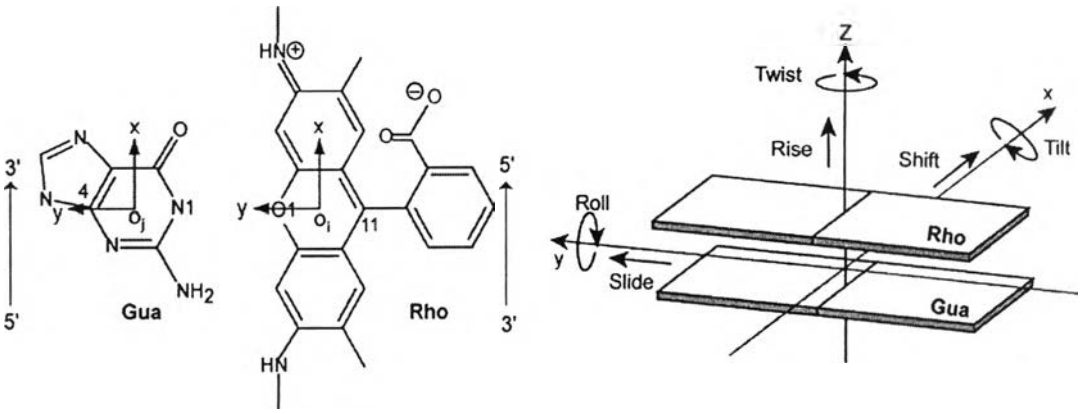

Figure 6.6 Coordinate system used to describe the position of the chromophore and the neighboring guanine base.

Table 6.1 Base-step parameters (translations in Å, rotations in degree) describing the relative position and orientation of rhodamine and guanine in the NMR structures where are rh6g1_28, rh6g1_38, tmr1_39, tmr2_20 and tmr2_3, derived by Griesinger *et al.*

System	Shift	Slide	Rise	Tilt	Roll	Twist
rh6g1_28	1.60	-3.57	4.95	-0.2	-1.2	-57.9
rh6g1_38	1.48	-2.72	4.82	-7.4	-8.6	10.5
tmr1_39	1.02	-1.98	4.44	-2.0	1.4	-72.3
tmr2_20	-0.21	0.87	4.26	-10.7	-27.0	36.6
tmr2_3	-0.35	0.71	4.34	1.4	-5.3	46.8

For this purpose, a rectangular coordinate system with its origin (o_i) at the midpoint between O1 and C11 and the y-axis points from C11 to O1 of Rho. (see Figure 6.6) For guanine, the y-axis lies along N1-C4. The origin, o_j, is given by the midpoint of the N1-C4. The z-axis is along the base normal and x-axis completes a right-handed triad set. The plane of Rho is fitted from 14 heavy atoms on the xantelium and gaunine plane is generated from 10 heavy atoms on this base. The step parameters were calculated by following the procedure of step parameter of DNA duplex. (see Chapter4)

The step parameters between Rho and guanine of five structures, rh6g1_28, rh6g1_38, tmr1_39, tmr2_20 and tmr2_3, derived by Griesinger *et al.* from NMR data are summerized in Table 6.1. In these structures, Rho was covalently attached to 5'-Cytocine of 20-mer. (Figure 6.1) This covalent is similar to 7-mer of Rho-5C system in our study. The structure of Rho in rh6g1_28 and rh6g1_38 is the same in present study (see Figure 6.1) but it is different in tmr1_39, tmr2_20 and tmr2_3 as shown in Figure 6.7.

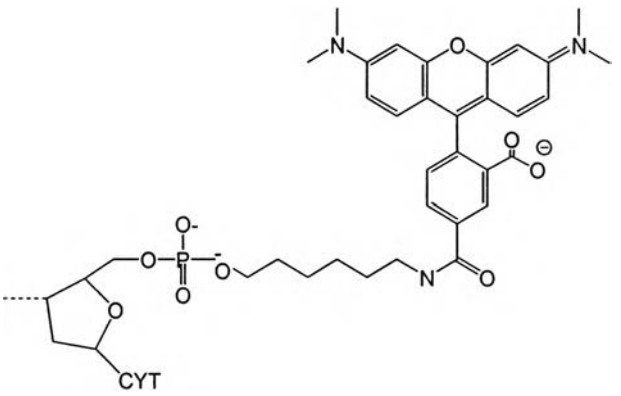

Figure 6.7 Rhodamine 6G derivatives in tmr1_39, tmr2_20 and tmr2_3 NMR structures

Note that the rise parameters which determine the π-stacking between the chromophore and guanine, are quite large, ranging from 4.20-4.95 Å as compared to the standard value in DNA, 3.4 Å. This holds especially in the cases rh6g1_28 and rh6g1_38 where as value of ~5 Å was found. At the distances longer than 4 Å, the π-stacking interaction should be very weak and no electron transfer is expected to take place (see Table 6.1).

The parameters tilt and roll characterize how well the planes of the chromophore and the guanine unit are held in a parallel arrangement. For all structures except tmr2_20 the angles are relatively small. Thus, both planes are approximately parallel.

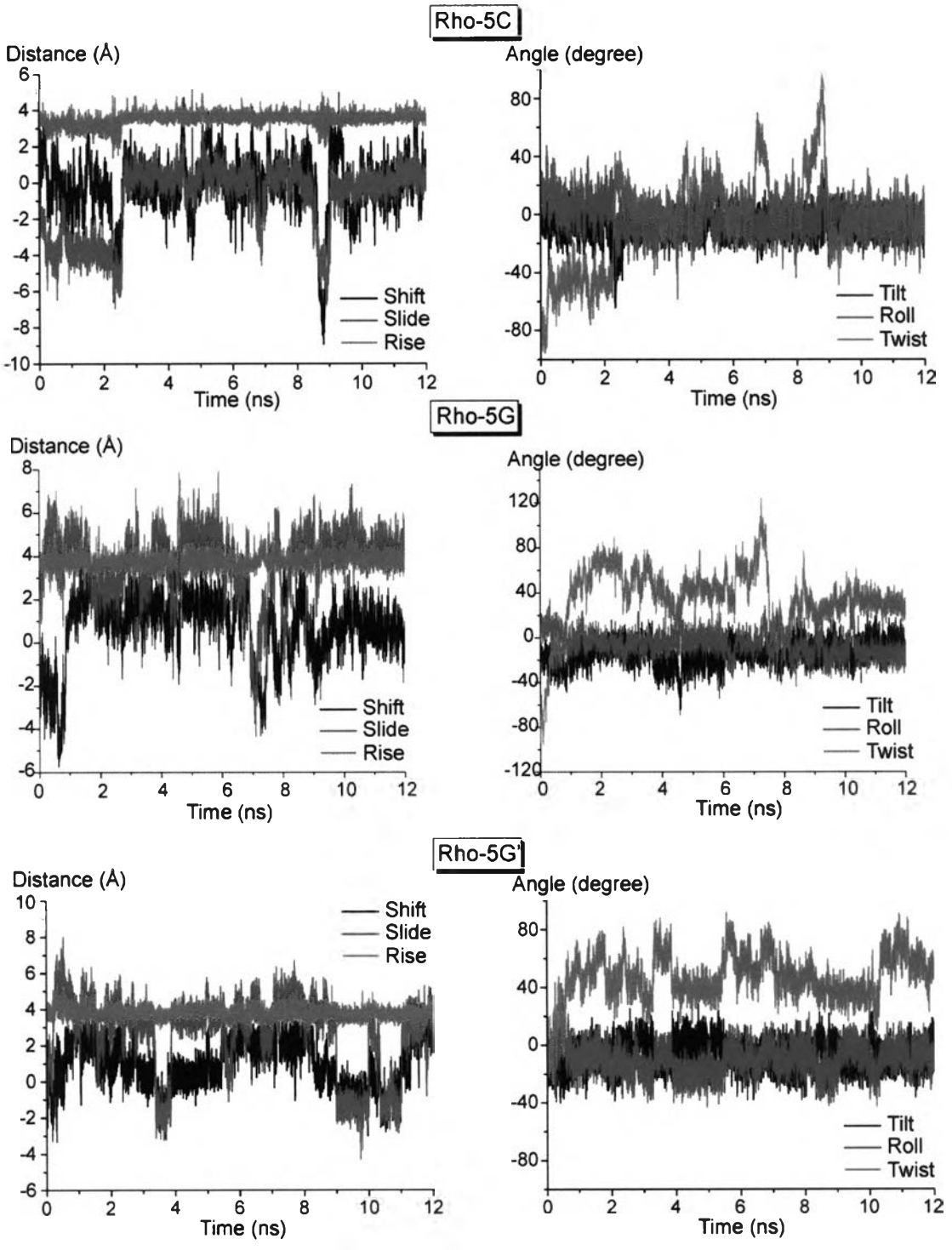

Figure 6.8 Results of MD simulations, demonstrating the fluctuations of the step parameters in the Rho–5C, Rho-5G and Rho-5G′ systems.

Because of the relatively large values for shift and slide in rh6g1_28 (4.95 Å) and rh6g1_38 (4.82 Å), these structures feature a considerable shift of the rhodamine moiety relative to the guanine unit. On the other hand, in the structures tmr2_20 and tmr2_3, the small value of the shift and slide parameters indicated that the chromophore is above the guanine base

In Figure 6.8 and in Table 6.2 the step parameters describing the mutual position of the chromophore and guanine in the duplexes Rho–5C Rho-5G, and Rho-5G' as obtained from the MD simulations were presented. As can be seen from the data, the chromophore position of all structures is very flexible: the slide and shift parameters vary considerably. However, the rise parameter changes only weakly in the MD trajectories. Actually, the standard deviations of slide and shift is about 1.5 Å whereas that of the rise is only 0.3 Å, similar to the findings between Watson-Crick pairs in MD simulations on DNA duplexes. [124] Therefore, the distance between the plans of the stacked chromophore and the GC base pair remains almost unchanged and fluctuates slightly around 3.6 Å, 3.8 Å and 3.8 Å in Rho–5C, Rho-5G and Rho-5G', respectively. Note that these values differ notably from the experimental NMR data where values as large as 4.8–5.0 Å have been determined. Also, the remarkable variations of the twist parameter, ranging from -2.5° to 47°, indicate that the Rho-DNA complexes feature very flexible structures where, however, the inter-plane distance remains essentially unchanged.

Table 6.2 Base-step parameters (translations in Å, rotations in degree) derived from MD trajectories of the model complexes.

System	Δτ (ns)	Shift	Slide	Rise	Tilt	Roll	Twist
Rho-5C	0-2.6	-0.6±1.6	-3.7±1.0	3.2±0.3	-8.2±12	7.0±12	-42.0±27
	2.6-12	0.0±1.6	-0.1±1.2	3.6±0.3	-8.3±6	-4.6±8	8.3±19
	0-12	-0.2±1.7	-0.9±1.9	3.5±0.3	-8.3±8	-2.1±10	-2.5±30
Rho-5G	0-12	0.8±1.6	3.9±1.6	3.8±0.4	-14.2±11	-5.63±10	35.9±27
Rho-5G'	0-12	1.1±1.3	3.0±1.8	3.8±0.3	-5.6±10	-10.5±11	47.0±16

On first glance, one may interpret the MD simulations of Rho-5C (Figure 6.8) such that they yield two distinct, but very flexible conformations. The first one is found during the interval from 0 to 2.6 ns as well as in the region close to 8.8 ns (Figure 6.9); the second structure seems to dominate the trajectory after 2.6 ns. We are currently analyzing these two types of conformations to characterize them in more detail and to identify their main features; however, already now we would like to add a few remarks.

The average parameters of these parts of the Rho-5C MD trajectory are quite distinctive (see Table 6.2). Inspection of Figure 6.8 reveals that the position of the chromophore in Rho-5C changes dramatically during 100–200 ps; for instance, in the time regions near 2.6 ns and 8.5 ns of the MD trajectory. Also the Rho-5G system shows considerable variations of the structure, *e.g.* near 1 ns and 7 ns (Figure 6.8).

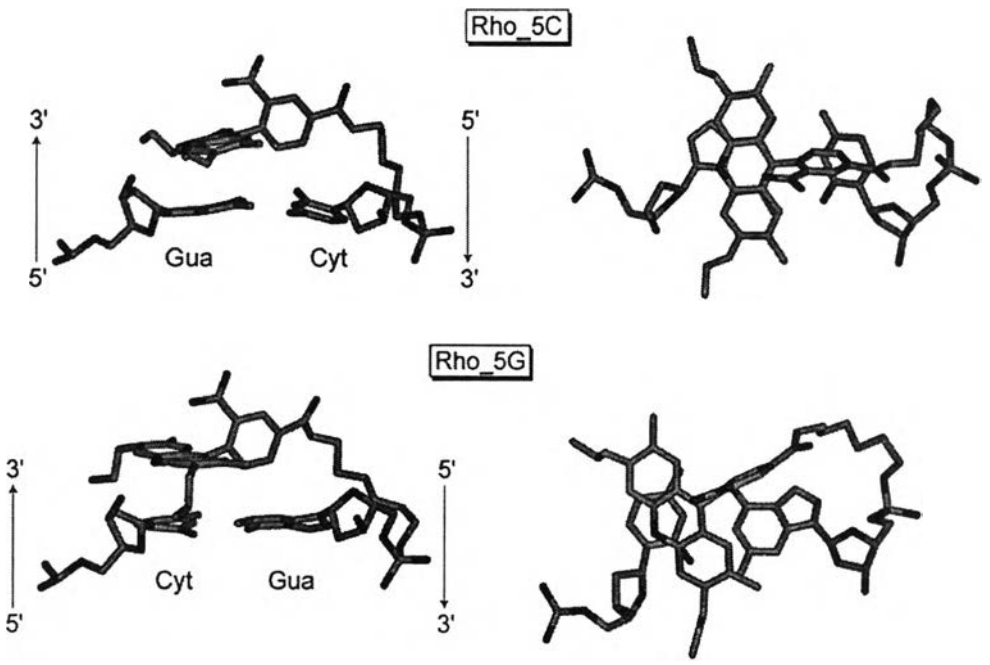

Figure 6.9 The average structure of Rho-5C and Rho-5G complex

Comparing the MD results, the characterized of Rho in Rho-5G and Rho-5G' are similar, which can be seen from rise and twist parameters in Table 6.2.Twist parameters are 35.9±27 and 47.0±16 for Rho-5G and Rho-5G', respectively. The average rise parameters are about 3.8 Å. They are larger than the value in Rho-5C because of the effect of the π-interaction between base and Rho. As can be seen, Figure 6.9 shows the average structure of Rho-DNA complexes. Average structure of Rho-5C illustrates that the middle ring of xanthene in the Rho is exactly above the six member ring of guanine base. This can see from the shift and slide parameters for 0.0±1.6, 0.1±1.2, respectively. While, the position of Rho is above between guanine and cytosine, the dye has covalent bond with guanine base. (Rho-5G and Rho-5G')

Taking into account our previous study on the sensitivity of the electronic coupling due to conformational changes of donor and acceptor sites, [61] we anticipate that the charge transfer rate will vary significantly with the position of the chromophore relative to the adjacent guanine. Because the position of the chromophore is very flexible, one is not able to determine the electronic coupling and the charge transfer rate on the basis of averaged geometries; rather, a strategy which relies on averages of pertinent characteristics along trajectories will be crucial.

In Figure 6.10 and Figure 6.11, we present the bond angles that describe the position of the selected linker (C26-C29) between chromophore and DNA. A linking atom is projected on the plane of base pair (point P_1). The corresponding angles, P_1-O-P2, are shown in Figure 6.11. As can seen, the linking atoms are very flexible.

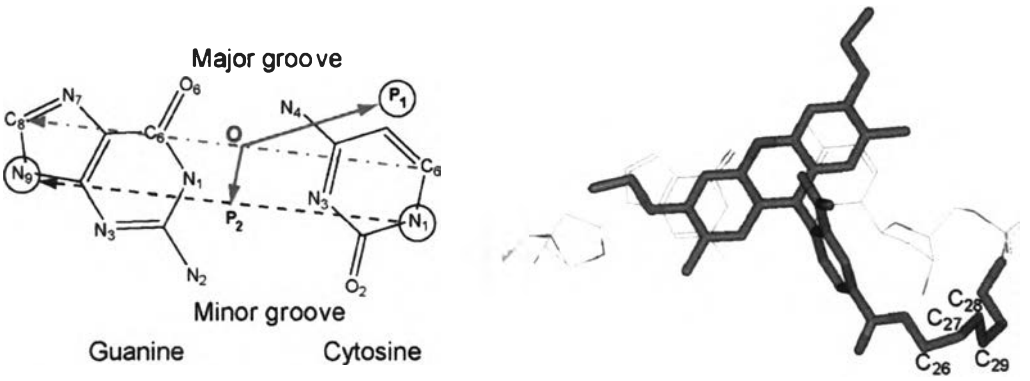

Figure 6.10 Schematic representation of the selected linker (C26-C29) between Rho and DNA where P1 is projection of a linking atom into (GC) plane, points O and P2 lye in the middle of C6 - C8 and N1-N9 distances, respectively

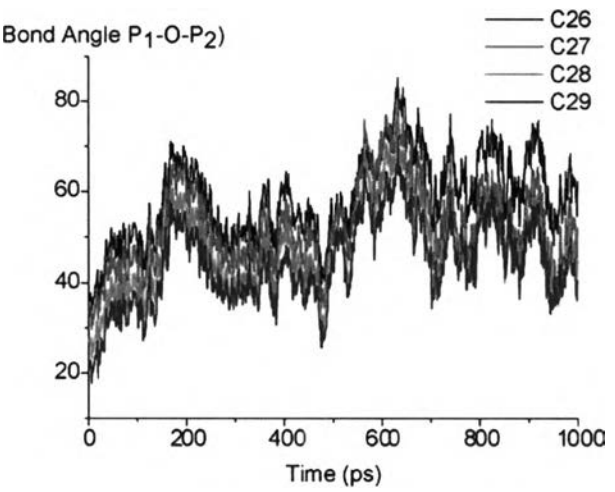

Figure 6.11 Position of linker atoms (the bond angle P_1-O-P_2 in degree) determined for MD trajectory of the complex Rho-5C within 1000 ps (from 11 ns till 12 ns)

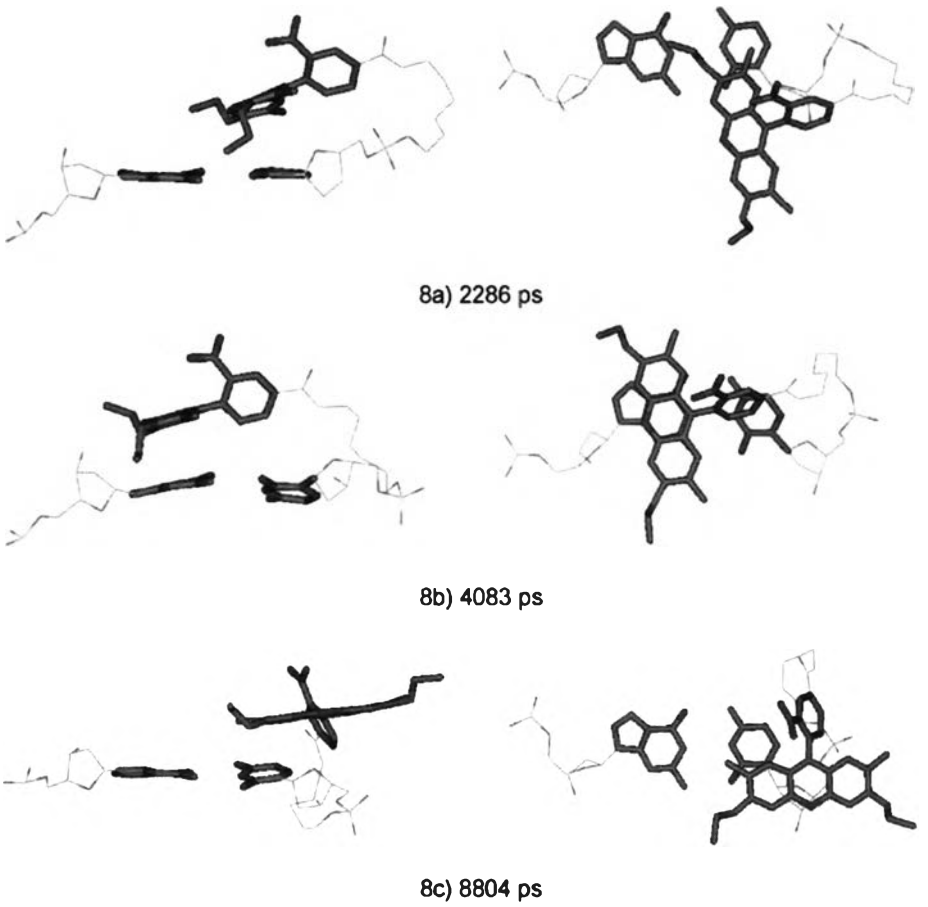

Figure 6.12 Selected structures of the Rho-C complex

Table 6.3 shows the step parameters of the chromophore and guanine in the Rho-5C complex (snapshots at 2286, 4083 and 8804 ps shown in Figure 6.12). The chromophore guanine in the structures 4083 ps and 8804 ps are nearly in parallel planes but in the structure 2286 ps they are not. In the structures 2286 ps and 8804 ps, rhodamine is considerably shifted relative the guanine base (see shift and slide parameters in Table 6.3)

Table 6.3 Step parameters between the chromophore and guanine in the Rho-5C system (translations in Å, rotations in degree) in three snapshots.

Time (ps)	Shift	Slide	Rise	Tilt	Roll	Twist
2286	-6.96	-5.03	3.23	-19.0	7.4	5.2
4083	-0.49	0.01	3.53	-3.8	2.1	3.4
8804	-8.57	-4.13	2.8	-5.3	6.9	90.2

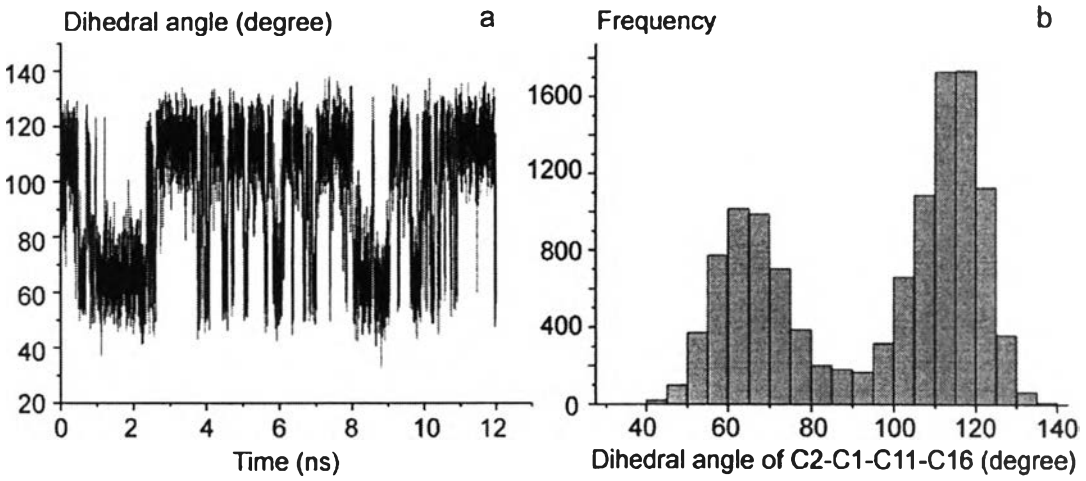

Figure 6.13 Fluctuation of the dihedral angle C2-C1-C11-C16 (defined in Figure 5.2) of Rho-C system along the MD trajectory a) its distribution and b) its histogram.

The dihedral angle C2-C1-C11-C16 was investigated along 12 ns and plotted in Figure 6.13. It was found that the angle is flexible and deviates from the optimized value. Two maxima of the distribution Plot (Figure 6.13b) of 66°±9° and 113° ±8° indicated the preferential conformation of the Rho-5C. This is as similar as the phenomena found in Rhodamine monomer in water the two conformers were observed of 70° ± 9° and 108° ± 9° (see Figure 5.13 in Chapter5)

6.2.2 Analysis of base pair dynamics

The position of base pairs DNA duplex can be characterized by local base-step parameters: shift, slide, rise, tilt, roll and twist which was again defined for the DNA base pair (Figure 6.14). Table 6.4 shows the average values and standard deviations of these parameters in the model Rho-5C. The program X3DNA has been employed to derive the parameters. The translation and rotation parameters analyzed wihtin MD trajectory change considerably (see line a in Table 6.4).

Interest is focused on the first base pair (5′-CA-3′) where cytocine base is directly connected to Rho. Discrepancies between simulated (a) and NMR data (b and c) for the shift and slide parameters in the first step pair, were considerably detected. The average value of rise parameter of 3.29±0.37 Å is in good agreement with that derived from NMR structure of 3.36 Å and 3.39 Å for rh6g1_28 and rh6g1_38, respectively. This is also true for the twist parameters of the first base-step shown in Table 6.4. For the others base-steps, consistency of the rise and twist parameters were observed.

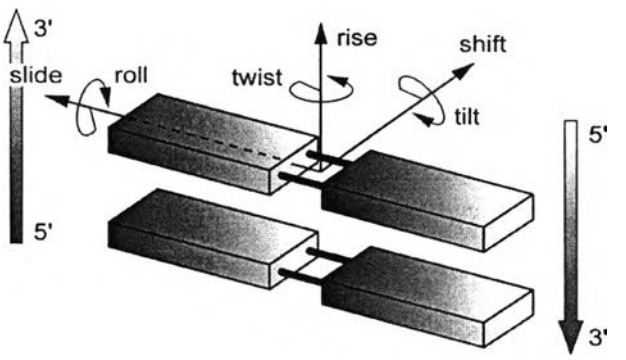

Figure 6.14 The six parameters used to describe the conformation of a base-pair in DNA

Note that, open structures for the last CG pair were seldom found. This is usually detected in almost all simulations. [161]

As it is know that the conformational fluctuations of DNA π-stacks has an effect on the electron transfer coupling. It was shown to be very sensitive to variations of the

mutual positions of the Watson-Crick pairs.[61] To seek for more information, base-step parameters for the A_2A_3 and A_3A_4 in Rho-p-5′-$CA_2A_3A_4$GCG-3′ complex (Rho-5C) were again summarized separately in Table 6.5. As can be seen, the average step parameters obtained from the present work are in good agreement with those of free DNA and ideal DNA duplex, *i.e.*, conformation and orientation of the AA second and third base pair were not be significantly disturbed by the stacking interaction with chromophore in aqueous solutions.

Table 6.4 Average base-step parameters between neighboring pairs in Rho-p-5′-CAAAGCG-3′ derived from the 12 ns MD trajectories a) and the NMR structure of rh6g1_28 (b) and rh6g1_38 (c).

Base-step		Translations (Å)			Rotations (degrees)		
		Shift	Slide	Rise	Tilt	Roll	Twist
CA	a	-0.26±0.62	-0.41±0.57	3.29±0.37	0.2±4.9	11.0±6.4	25.6±6.7
	b	-0.36	-1.27	3.36	1.2	11.7	23.8
	c	0.96	-1.00	3.39	-0.5	8.2	26.3
AA	a	-0.59±0.66	0.02±0.53	3.34±0.29	-2.9±3.9	2.7±5.3	36.9±5.7
	b	-0.32	-0.48	3.34	1.1	-4.4	32.9
	c	-0.04	-0.34	3.36	-1.2	-3.7	34.9
AA	a	0.09±0.59	-0.38±0.51	3.31±0.29	-2.7±3.9	2.6±5.1	34.3±5.1
	b	0.44	-0.71	3.39	2.4	-3.2	37.4
	c	-0.05	-0.76	3.33	0.8	-3.7	34.8
AG	a	-0.40±0.65	-0.89±0.54	3.50±0.30	-3.3±4.3	4.2±5.1	35.3±5.3
	b	-0.64	-1.13	3.31	0.2	-3.4	30.9
	c	0.26	-0.63	3.34	1.1	-3.7	35.2
GC	a	0.19±0.68	-0.48±0.58	3.44±0.28	1.3±4.3	-0.2±4.8	36.9±4.5
	b	0.38	-0.25	3.35	2.1	-4.0	40.2
	c	-0.13	-0.36	3.34	-1.3	-3.7	37.0

Table 6.5 Base-step parameters of 5′-AA-3′ obtained from MD simulation of Rho-p-5′-$CA_2A_3A_4GCG$-3′

	Translations (Å)			Rotations (degrees)		
	Shift	Slide	Rise	Tilt	Roll	Twist
A_2A_3	-0.59±0.66	0.02±0.53	3.34±0.29	-2.9±3.9	2.7±5.3	36.9±5.7
A_3A_4	0.09±0.59	-0.38±0.51	3.31±0.29	-2.7±3.9	2.6±5.1	34.3±5.1
AA^*	0.01±0.49	-0.93±0.57	3.43±0.23	-1.4±2.4	1.7±4.2	30.4±3.6
Ideal structure**	0.00	0.00	3.4	0.0	0.0	36.0

6.3 Conclusions

In conclusion, the results of MD simulations for the three systems, Rho-5C, Rho-5G and Rho-5G,' indicate that rise parameter changes only slightly in the MD trajectories. The distance between the planes of the stacked chromophore and the GC base pair remains almost unchanged and fluctuates around 3.6 Å and 3.8 Å in Rho–C and Rho-G, respectively. The variations of the twist parameter indicate highly flexible of the chromophore-DNA complexes.

For further work, the obtained MD trajectories for the three system should be used to study the charge transfer rate is supposed to vary significantly with the position of the chromophore relative to the adjacent guanine, so the electronic coupling and the charge transfer rate cannot be determined on the basis of averaged geometries.