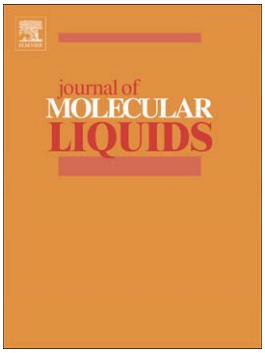

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Molecular dynamics simulation of a DNA duplex labeled with triarylmethyl spin radicals

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Abstract

All-atom molecular dynamics simulation of aqueous solution of DNA duplex with triarylmethyl (TAM) spin labels attached to its ends through piperazine linkers has been carried out. The dynamics and structure of the resulting molecule on time scales up to 2 microseconds has been studied. Two classes of specific conformations have been observed. In the first one, a duplex retains its structure, and the labels on the average are arranged above the end bases of the duplex. Another class is related to the appearance of fraying at the duplex ends, i.e. involves situations where bonds between the end bases are broken, and the end pair is open. In this case, the labels get additional opportunities for the movement. Furthermore, due to torsional rotations in the linker, large deviations from the equilibrium states are possible. It is discussed the detected fraying of duplex labeled with TAM is not the inevitable result of the impact of labels. Our additional simulation has shown that fraying also occurs in the duplex without labels. Thus, despite its size, the TAM spin label does not critically influence the structure of the duplex. The distribution of distance between the labels for conformations of duplex without fraying has been shown to be in good agreement with the experimental distribution measured by pulsed EPR for the same molecule immobilized on a substrate in an aqueous solution.

Keywords: Molecular dynamics simulation, Triarylmethyl (TAM) radical, DNA duplex, DNA fraying

1. Introduction

Incorporating a probe molecule (fluorescence or spin label) into a protein or DNA followed by measurement of the structure and dynamics of biomolecules, is extensively used in a variety of experimental techniques, including fluorescence spectroscopy, electron paramagnetic resonance (EPR), and Forster resonance energy transfer (FRET) measurements [1-3]. However, the question arises, how the presence of the probe perturbs the local structure and dynamics when an unnatural probe molecule is inserted into a biomolecule [4,5]?

It is difficult to answer this question experimentally. However, the behavior of labeled and unlabeled molecules can be compared using molecular dynamics simulations. To a large extent, this question concerns the fluorescent labels (dyes) that are rather large. Special studies having been conducted, for example, in [5] authors have shown that the assumption necessarily made for

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all spectroscopic measurements, that the dye does not severely influence protein dynamics is justified. They calculated the root mean square fluctuations of the protein backbone for the protein with the bound dye and without the dye. Thus the perturbation was quantified and found to be small.

Nitroxide radical is widely used as a spin label in experiments with pulsed dipolar electron paramagnetic resonance spectroscopy [2,6-10]. It is quite small and its influence on the molecule is not usually discussed. However, some problem arises when large spin labels are used. Such labels are interesting due to the fact that it may be possible with their help to carry out the EPR experiments with biological systems under conditions close to the physiological ones. An intensive search for new spin labels with optimized relaxation properties has been carried out during last several years [11-13]. One of such large spin labels is triarylmethyl (TAM) radical having the correlation time (T_m) on the order of microseconds in liquids at room temperature [14]. This profoundly long relaxation makes it a promising alternative for nitroxide spin labels. Recently TAM radicals have been successfully applied for distance measurements at cryogenic [15,16], and at room temperature in liquid water for DNA duplex immobilized on solid sorbent [17].

Additional interest in studying possible perturbation of molecular structure with attached TAM radicals is concerned with the fact that this label is used to study DNA molecules. In contrast to proteins, DNA duplex can undergo additional structural changes associated with the opening of the terminal pairs. This process is often referred to as «fraying», which occurs for pairs of complementary bases. Study of this phenomenon is the subject of many experimental investigations [18-21], as well as of theoretical ones and of computer simulation [22-29].

In particular, NMR experiments have provided valuable information on the thermodynamics of this process [18]. The frayed states are enthalpically unfavorable, but they are stabilized entropically. Unfortunately, no definite information as to the structure of the frayed state could be extracted from the NMR experiments due to its flexible nature. In Ref. [27] the 100-ns molecular dynamics simulations of a DNA duplex with explicit salt water at 400 K was performed to clarify the mechanism of the melting transition of DNA. Several relevant motions and conformational states were observed. In Ref. [28] a large-scale all-atom simulation of Dickerson-Drew dodecamer duplex was carried out whose structure and dynamics have been investigated by many experimental and computational studies. Simulation was done with explicit representation of water and ions. The 0.6 − 2.4-μs-long MD trajectories were obtained and analyzed. In particular, it was found that the duplex ends can adopt an alternative base-pairing, which influences the oligomer structure. The fraying of the terminal base pairs of DNA and RNA duplexes was examined using intensive molecular dynamics simulations in Ref [29]. Analysis of microsecond time scale trajectories showed frequent disruption of the terminal base pair, exposure of the bases to solvent, and formation of stable noncanonical structures. These structures usually appear within the first tens to hundreds of nanoseconds. It was noticed that some features of fraying seemed to be consistent with the available experimental results. However, potential problems of the force field being used were pointed out.

It can be noted that all-atom simulation allows one to study in detail the ongoing structural transformations. However, it is insufficient for obtaining reliable sampling while working with complex molecules. The lifetime of some conformations is comparable, or even longer than the simulation time. To obtain a sufficient statistics one has to use enhanced sampling molecular dynamics methods or specialized high-performance computer systems, as discussed in the papers [30-33].

It remains unclear to what extent spin label attached to the duplex affects its stability. In this study, all-atom MD simulation of an aqueous solution of DNA duplex labeled with two TAM radicals was conducted and compared with the original duplex under the same conditions.

2. Method

We study the molecule being a B-DNA duplex labeled with two TAM radicals, which had been synthesized and experimentally investigated in [17]. Duplex consisted of 10 pairs of sequence 5'-CACGCCGCTG-3' / 5'-CAGCGGCGTG-3'. TAM labels were attached via piperazine linkers. Each linker was attached via a carboxyl group to the 5'-oxygen atom of the duplex and the other end was connected with the TAM carboxyl group, Fig. 1.

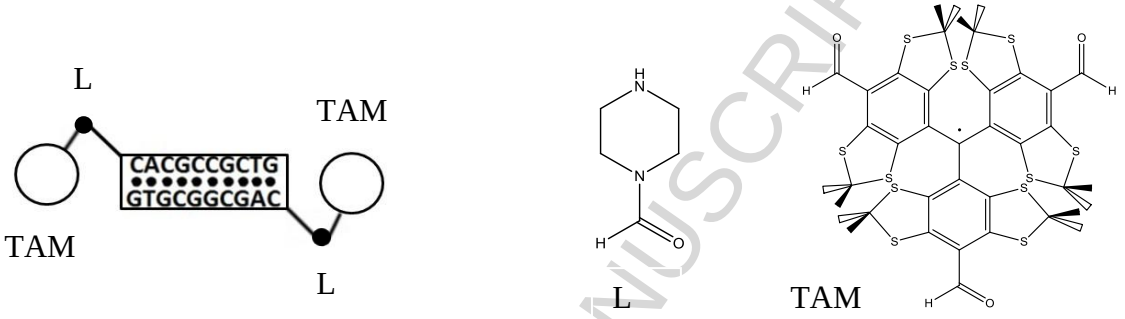

Fig. 1. (Left) the molecule studied (schematic), TAM radicals attached to the 5'-ends of the DNA duplex through the piperazine linkers. (Right) piperazine linker (L) and a triarylmethyl radical (TAM).

Amber force field ff14SB [34] was used to model the DNA because of its accurate representation of nucleic acids. Atomic coordinates and duplex topology file were obtained using the NAB utility from AmberTools package [35]. TAM radical and a linker used were lacking in the library of standard molecules of Amber package. So, to obtain them we have conducted a separate parameterization using Antechamber tools and the GAFF universal force field [36]. For this purpose, TAM radical was connected to the linker, and the free end of the linker was closed with capping methoxy group. Then according to the standard parametrization procedure ROHF / 6-31G* RESP charges were calculated for this fragment. While balancing the charges the charge +0.1896 was fixed on the cap's methyl group to accurately compensate for the charge of the terminal -OH group of DNA, in place of which a linker with radical is attached. Missing cross force-field parameters at the joint between DNA and L-TAM were deduced from both ff14SB and GAFF force-fields in consistent manner. The resulting molecule was placed in a box with periodic boundary conditions (7.2 x 8.0 x 8.3 nm) surrounded by 15236 molecules of TIP3P water [37]. The size of the box was chosen to guarantee a sufficient distance between the molecule and its periodic images during the simulation. 18 Na+ counterions were added for the electrical neutrality of the system. After that, the topology and the coordinates of the system have been converted using the ACPYPE utility [38] into the GROMACS package format, with which all the molecular dynamics simulation were carried out [39].

The simulation was performed in the NPT ensemble with a pressure of 1 bar at temperatures of 283 K and 310 K. Parrinello-Rahman barostat [40] and V-rescale thermostat [41] were used for pressure and temperature coupling. (See .mdp files with simulation parameters in Supplementary material). Two independent trajectories, each being of length 2 μs were calculated for TAM-labeled duplex at 310 K. The trajectory was of 800 ns for the temperature of 283 K. To simulate a single duplex (unlabeled) a box of size 6.8 x 6.7 x 7.8 nm with 11412 water molecules inside was used. In this case, simulation time was limited to 500 ns. VMD software was used for visualization of molecular conformations [42].

3. Results

3.1. Distances between TAM radicals

To characterize the conformations of the labeled duplex we calculated distances between the centers of the TAM radicals, R_{12}, and the separation between the radical centers and the center of duplex, R_{1C} and R_{2C}, Fig. 2.

The central carbon atom defines the center of TAM radical naturally. It has maximal spin density, and the distance between these atoms is measured in EPR experiments. The center of mass of group of atoms from the middle of the both chains was selected as the center of the duplex. Atoms O2, N3, C2 from the base DC5 and atoms N3, C4, N4 from the base DC6 were selected on the A chain. Atoms H1, C6, N1 from the base DG5 atoms N2, C2, N1 from the base DG6 were selected on the B chain. The center of mass of this group of atoms does not undergo significant fluctuations as individual atoms or bases. In addition, it is little affected by the movement of radicals. Therefore, the distance between this center and the center of radical characterizes the radical position relative to the duplex.

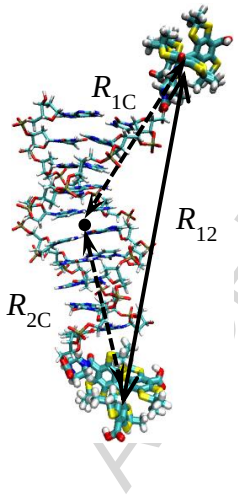

Fig. 2. The distances used for characterization of the labeled duplex conformations. Solid arrow (R_{12}): between the centers of TAM radicals, dashed arrows (R_{1C} and R_{2C}): between the centers of radicals and the center of duplex (see text).

Fig. 3 shows the change of the specified distances in time for the model with a temperature of 310 K. MD trajectory of duration 2 μs was used. The behavior of the curves over time is very complex. Against the background of "uniform noise" with typical amplitude about 0.5 nm, considerable spikes are observed, with the value of R_{12} being changed by more than 1 nm. Such spikes occur once about every 100 ns, and the upward movement usually accompanied by the downward one. It is interesting that the duration of these extreme deviations is of nanoseconds, that is, they correspond to real conformations. Actually, shown in Fig. 4 is an enlarged section of the curve for R_{12}, marked in Fig. 3 as rectangle. The behavior of the system during such a spike is seen to have a “diffusive” nature, i.e. TAM radicals perform complex movements while being in the extreme position.

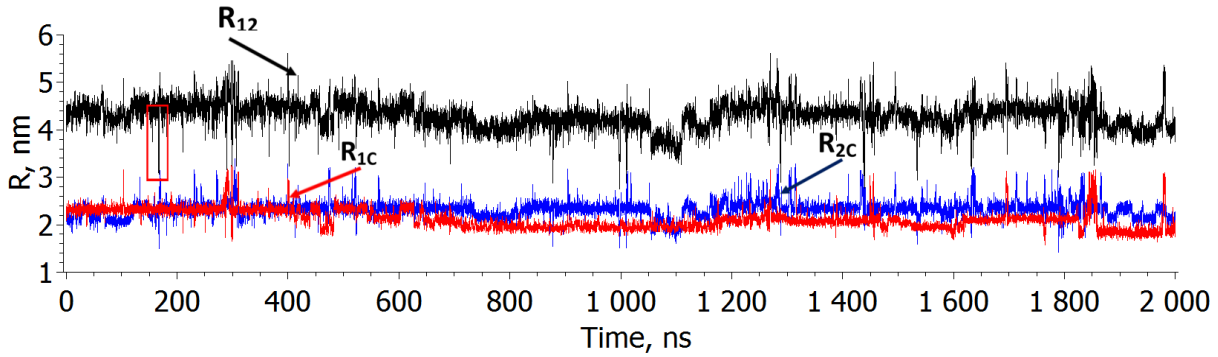

Fig. 3. Time behavior of the separation R_{12} (black) between the TAM radicals, and between the radicals and the center of duplex R_{1C} and R_{2C} (red and blue) during 2 μs production run simulation. The narrow peak near 170 ns (marked by rectangle) is shown in Fig. 4 in larger scale.

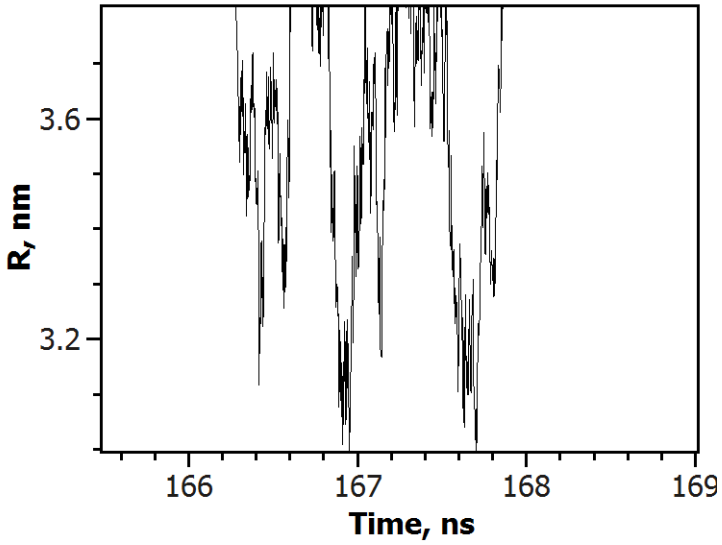

Fig. 4. The part of the curve for R_{12} distance, marked by a rectangle in Fig. 3, in enlarged scale.

Besides the marked "high frequency noise", deviations from the mean distance with duration of tens or hundreds of nanoseconds are observed. The nature of these deviations will be discussed below.

Curves for R_{1C} and R_{2C} demonstrate behavior similar to the curve R_{12}, Fig. 3. All the characteristic features appear at the same points of time as on the curve R_{12}. This is natural, since the distance between the radicals and the distance between the radicals and the center of the duplex are the dependent variables. However, the curves R_{1C} and R_{2C} do not coincide with each other. This means that the TAM radicals on different ends of duplex behave independently.

3.2. Distances between the bases of terminal pairs

To study the conformational changes in the duplex in more details we additionally monitored the behavior of the terminal bases. Throughout the molecular dynamics trajectory we calculated the distance between the centers of the terminal pair bases D_{12} and the distances from the center of the duplex found earlier to the centers of these bases, D_{1C} and D_{2C}, Fig. 5. Atoms N4 (in the base DC1of chain A) and N1 (in DG10 of chain B) were chosen to be the centers of the bases. Similar distances can be defined for the second end of the duplex: D'_{12} - between the centers of the terminal pair of bases, and D'_{1C} and D'_{2C} - between the centers of the bases and the center of the duplex. Here, atom C2 in base DG10 for chain A and atom N4 in the base DC1 for the chain B were chosen as the centers of the bases.

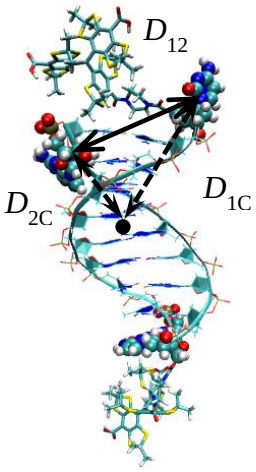

Fig. 5. Additional distances for characterization duplex conformations. Solid segment D_{12} is between the centers of the terminal bases, dashed segments D_{1C} and D_{2C} are between the centers of the terminal bases and the center of duplex (see text).

Time dependence of these distances for the same molecular dynamic trajectory is shown in Fig. 6 for the both ends of the duplex. We see a typical “fraying” of the duplex. Sometimes when terminal pair is opened: the distance between the bases increases. In a few tens or hundreds nanoseconds they can come back to the normal state but sometimes the behavior is more complex.

For the first end of the duplex, the value D_{12} increases time to time, sharply from 0.5 up to 1.0 or even 1.5 nm, and after a period of time it comes back, see blue line in Fig. 6(1). The curves D_{1C} and D_{2C} reveal here, that only one base of the terminal pair moves away from its initial position. Only D_{1C} curve demonstrates jumps, which correspond to the jumps of base-base distance D_{12}. The second base keeps the original distance to the center of the duplex during the entire simulation period: value D_{2C} (orange line) fluctuates slightly around its original value 1.5 nm without any jumps.

The other end of the duplex demonstrates different behavior, Fig. 6(2). After 650 ns of simulation, this terminal pair was opened, and the both bases changed their locations: one turned out farther from the center, and the other became closer, see D'_{1C} and D'_{2C} curves. As a result, the distance between the bases D'_{12} increases up to 2.5 nm, what is much more than we saw on the first side of the duplex. This type of conformations existed during a half of microsecond. Then the structure changed, but it did not come back to the original state up to the end of the production run. We see completely different set of distances at the rest of the trajectory, Fig. 6(2).

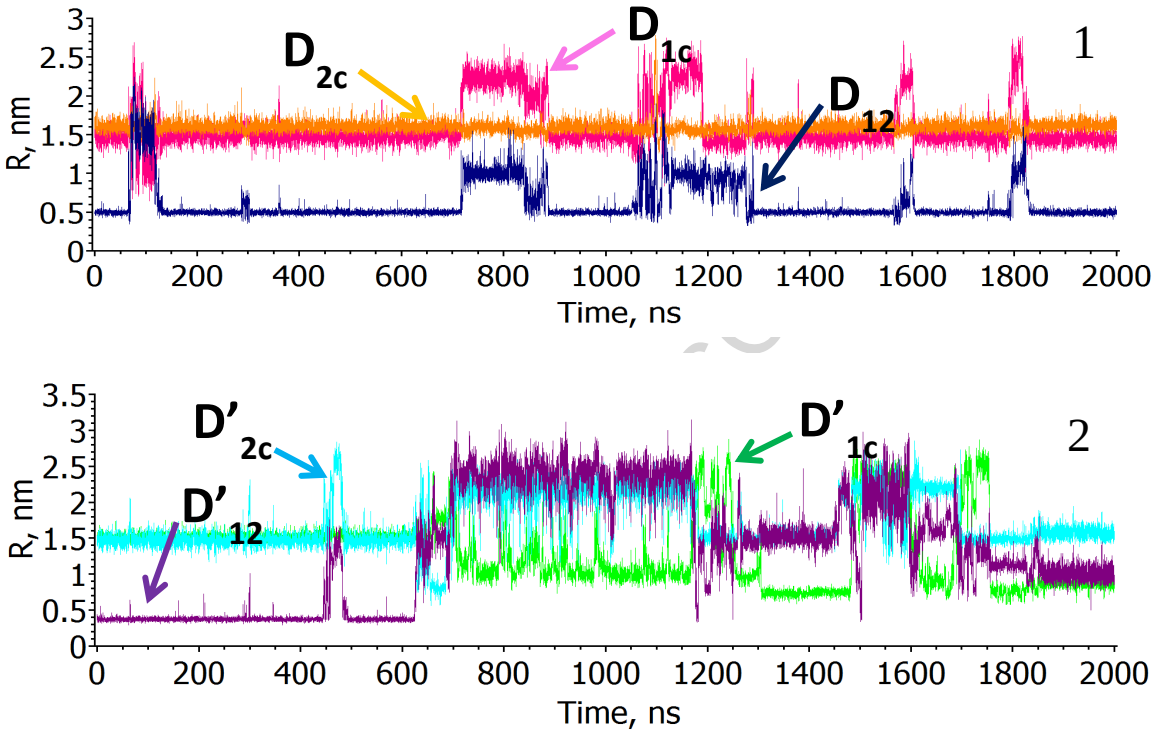

Fig. 6. Time behavior for the bases of terminal pairs in the duplex labeled by TAM for the trajectory presented in Fig. 3. (1) - the first end. D_{12} is the distance between the centers of a terminal pair bases. D_{1C} and D_{2C} are the distances between these centers and the center of duplex, see text. (2) - the second end. D'_{12}, D'_{1C}, and D'_{2C} are the corresponding distances.

3.3. Groups of conformations

Thus, we see two main groups of conformations of the labeled duplex. The first one can be called *normal* conformations. In this case, the duplex is intact, i.e. there are no open terminal pairs. As discussed in Ref. [29], there is a variety of conformations of the terminal pairs, which are different from the canonical Watson-Crick structure. We cannot see many details of the structure using only the distances for structure characterization, but we can say that the DNA duplex is unbroken at least for these conformations. The other group can be called *defective* conformations.

Some examples of the *normal* configurations of the labeled duplex are shown in Fig. 7. In this case both radicals are capping the terminal base pair [17]. We think that these conformations are stabilized by hydrogen bonds between carboxyl groups of TAM and ribose ring on the 3'-end of complementary chain. During the visual inspection of the trajectory, we constantly observed the formation of such bonds. Both terminal bases here are usually isolated from water molecules.

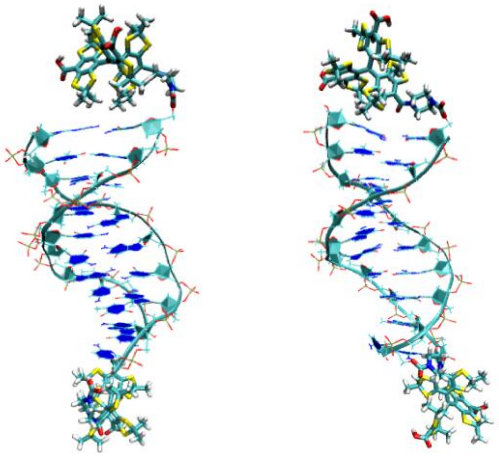

Fig. 7. Example of *normal* conformations of DNA duplex with attached TAM radicals. The duplex is intact. Both radicals are capping terminal base pairs.

Examples of the *defective* conformations are shown in Fig. 8. Their diversity is substantially greater than that of normal conformations. They give a wide variety of R_{12} distance values, which are usually lower than for normal structures. It often happens that diverged bases form pair again and defective configuration turns into normal. However, there are quite stable configurations, e.g. the one shown on the right side in Fig. 8. It existed in our simulation for the hundreds of nanoseconds and persisted until the end of the simulation. In this conformation the terminal base from the TAM-containing chain turned sideways, and the second base of the pair laid in the groove of the duplex, while the radical lowered down and took a place of the unzipped pair.

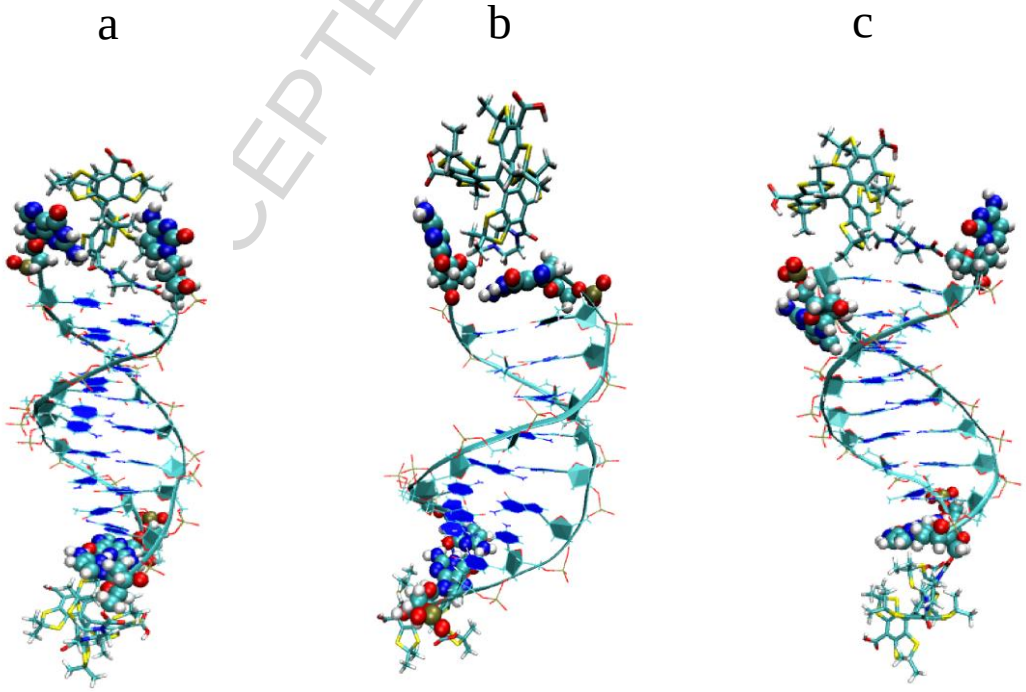

Fig. 8. Example of the *defective* conformations of DNA duplex with attached TAM radicals. One or both of terminal base pairs of a DNA duplex are open (terminal bases are shown as van der Waals spheres). Left to right: (a) the radical is "squeezed" between the bases of open terminal pair, (b) the radical is connected to one of the bases by the hydrogen bonds, (c) one of the bases lies in the DNA groove while the radical has lowered into place of the open pair.

As discussed above, sometimes R_{12} distance undergoes abrupt fluctuations, see spikes in Fig. 3. Example of corresponding conformations is shown in Fig. 9. Detailed analysis has shown that extremely large and extremely small distances have a common origin, namely, the torsional twists in the linker. At first, due to such twist, radical leaves its normal position and appears on the large distance from the center of the duplex. However, further rotation is possible in the linker, resulting in the radical being rotated by a large angle and being found near the side surface of the duplex. The distance between radical and the center of the duplex (and also between radicals) becomes, on the contrary, small. However, the lifetime of such conformations is not more than a few nanoseconds, see Fig. 4. We call such situations *extreme conformations*. Note that they happen both in the normal state of a duplex and in the defective one (see Fig. 3 together with Fig. 6). It is understandable, because they are caused by the linker.

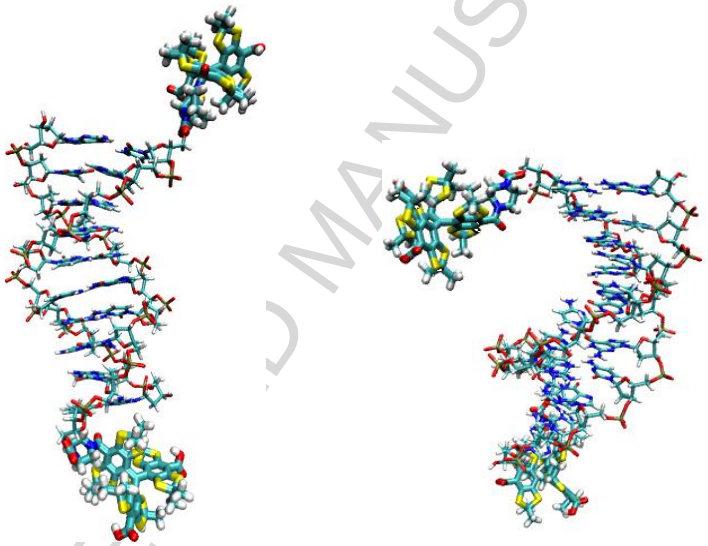

Fig. 9. Example of the extreme conformations of DNA duplex with attached TAM radicals. On the left: one radical is displaced from its initial position due to torsional rotation in the linker. On the right: after the subsequent rotation in the linker the TAM radical can appear at the side of the duplex.

An independent run of our model lasting 2 μs gives the same qualitative results, i.e. there are normal, defective and extreme configurations in approximately the same quantity. The difference is that they appear in moments, different from the first run.

The modeling at the lower temperature (283 K) also gives similar results, only with the small differences, which are natural for temperature decrease. There is a minor decrease of the noise fluctuations: while in Fig. 3 typical deviations of R_{12} is about 0.5 nm, now it has reduced to 0.4 nm. The extreme configurations become less frequent. The duplex fraying is also observed, but to a lesser extent: during 0.8 μs simulation only one terminal pair was open for approximately 400 ns, and restored to the normal state to the end of the run.

3.4. DNA duplex without TAM

To answer the question whether the presence of *defective* conformations is caused by TAM labels or not, we carried out the MD simulation of the duplex without labels at the same temperatures, 283 K and 310 K. It was found that fraying is observed at both temperatures already for the trajectory length of 0.5 μs. It is interesting that at the temperature of 283 K one base pair opened already after 50 ns, see Fig. 10. Then, after strong structural fluctuations during the next 50 ns, one of the bases turned sideways, laid down into the duplex groove and became closer to the center of the duplex, while the second base remained at the original distance from the center. The resulting structure was not changed until the end of the simulation, Fig. 10 (1). At the other end of the duplex, a terminal base pair was experiencing small reversible fluctuations during 300 ns and then a stable configuration appeared, which was preserved until the end of the trajectory. At the same time, this terminal pair did not open during the simulation, Fig. 10 (2).

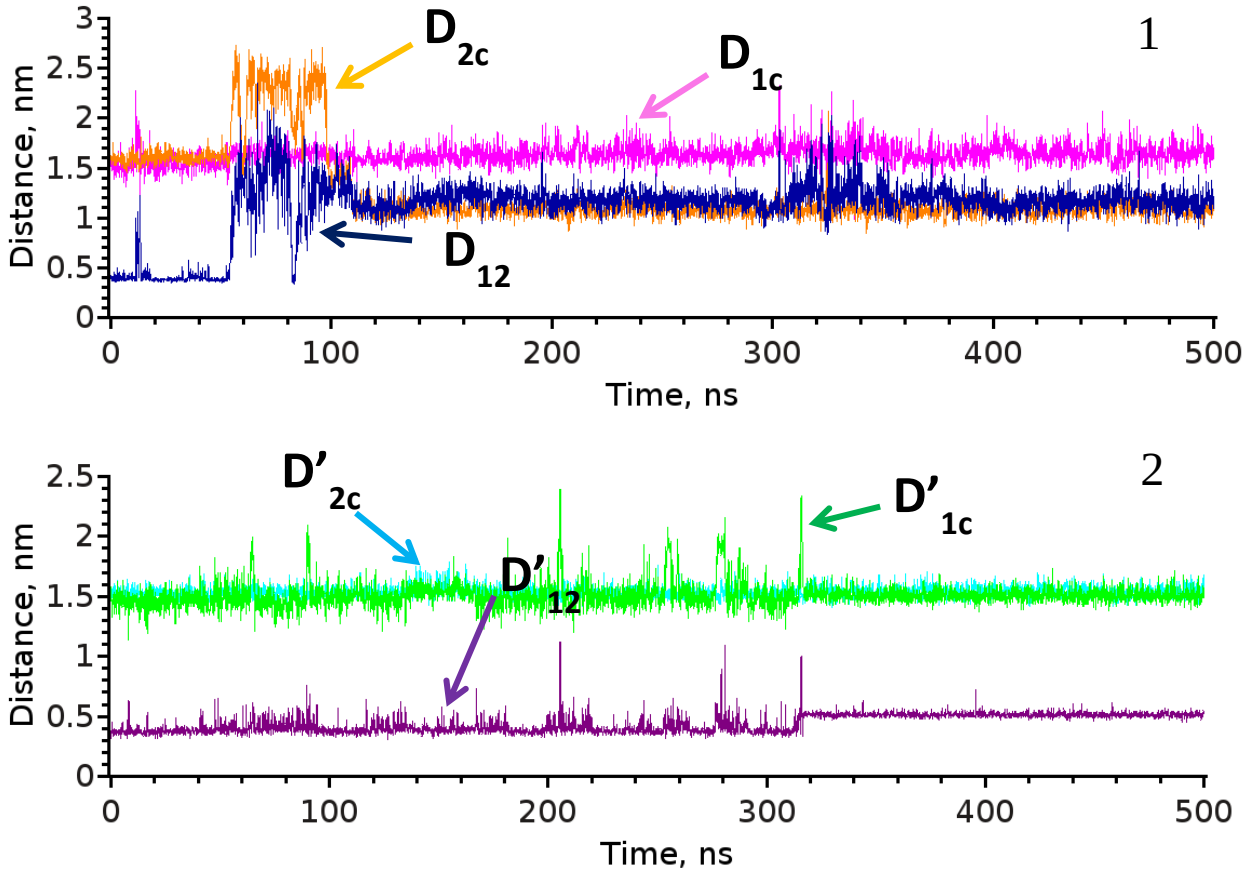

Fig. 10. Time behavior for bases of the terminal pairs in the pure duplex in water at 283 K. (1) - the first end. D_{12} is the distance between the centers of a terminal pair bases. D_{1C} and D_{2C} are the distances between the centers of the bases and the center of the duplex, see text. (2) - the second end. D'_{12}, D'_{1C}, and D'_{2C} are the corresponding distances.

The fraying was also found at 300 K, It happened on the second end of the duplex after 400 ns of simulation, Fig. 11 (2). There were noticeable fluctuations before this moment, but the pairs did not open.

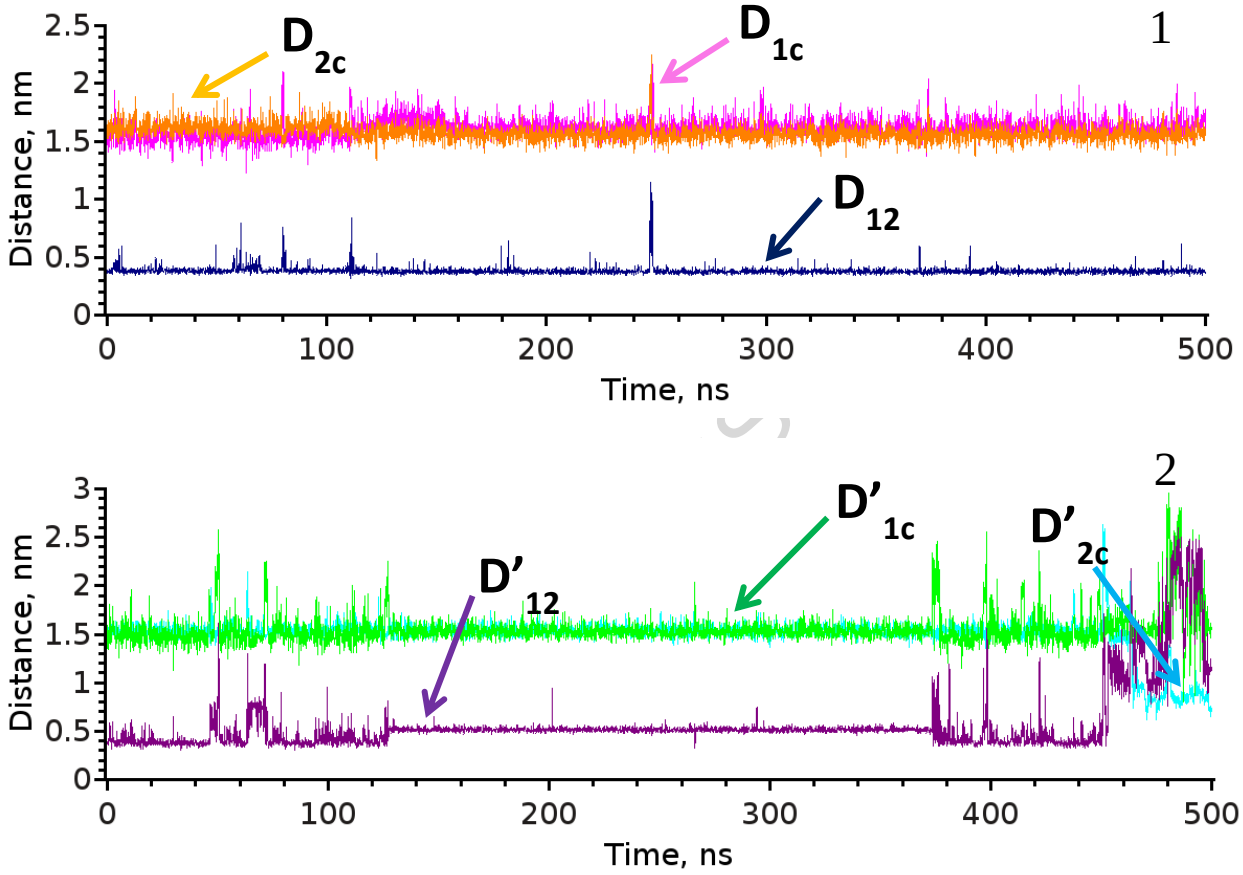

Fig. 11. Time behavior for bases of the terminal pairs in the pure duplex in water at 310 K. See Fig. 10 caption.

The other end of the duplex seems even more stable than at a lower temperature, compare Fig. 11 (1) and Fig. 10. We think, it is related to insufficient sampling of the classical all-atom simulation. Nevertheless, it can be said that fraying is inherent to the duplex and this phenomenon is not a result of the presence of TAM labels.

3.5. Distance distribution between TAM radicals

Fig. 12 shows distribution of distance R_{12} between centers of TAM only for *normal* conformations at 310 K. We used our 2 μs production run, Fig. 3. Recall that *normal* conformations are those that have both terminal base pairs closed. To select them we used the curves in Fig. 6.

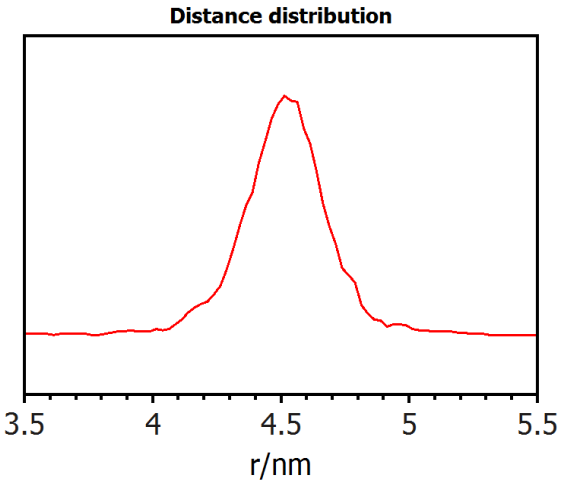

Fig. 12. Distance distribution between the centers of TAM spin labels calculated for *normal* conformations of the duplex (see text) for the trajectory represented in Fig. 3.

This distribution is rather close to the experimental one obtained in [17] for the same duplex with TAM immobilized on a sorbent in aqueous solution. The only difference is that the position of the maximum of the distribution (4.50 nm) is slightly shifted towards lower values compared with the experimental ones (4.52-4.61 nm). This difference may be due to the lack of some distorted conformations in the experiment. The duplex can be more stretched on the sorbent than in bulk water. Molecular dynamics simulation that was also carried out in [17] shows somewhat higher value for the position of the maximum (4.64 nm). One may explain this by the insufficient sampling (they used 200 ns production run) or by the force field used. However, all these deviations lie within the experimental error.

Note that the distance distribution calculated for the whole trajectory (including *defective* conformations) would be uninformative because classical all-atom molecular dynamic cannot provide sampling for such complex systems even for microseconds scale simulation.

4. Conclusions

All-atom molecular dynamics simulation of the DNA duplex molecule labeled with two triarylmethyl (TAM) spin radicals in aqueous solution was carried out. Conformations of this molecule were compared with conformations of duplex without radicals. It was shown the presence of fraying of DNA duplex: the terminal pairs of the duplex can be open and their bases can be moving independently. Sometimes the duplex recovers its structure after tens or hundreds of nanoseconds. However, some frayed conformations persist for longer time. Since the duplex fraying is manifested both with the presence of the labels, and without them, we assume that the presence of TAM radicals have no drastic impact on the duplex. It is important for the use of this radical in EPR experiments as a spin label for DNA.

Interestingly, the distribution of distances between TAM radicals calculated in our simulation for normal configurations (without duplex fraying), is in a good agreement with the experimental data obtained by pulsed EPR for this molecule immobilized on a sorbent in water [17]. The only difference is that the maximum of the calculated distribution is slightly shifted to smaller distances. However, it can be said that this difference is within the accuracy of the experiment. It means that the labeled duplex on the sorbent does not contain opened terminal pairs. Indeed, the immobilization can restrict motions of the molecule, and some conformations become impossible.

The comparison of different simulation force fields is of special interest and requires additional study. For the reference, we provide our simulation parameters and molecular topology files in *Supplementary material.*

Acknowledgements

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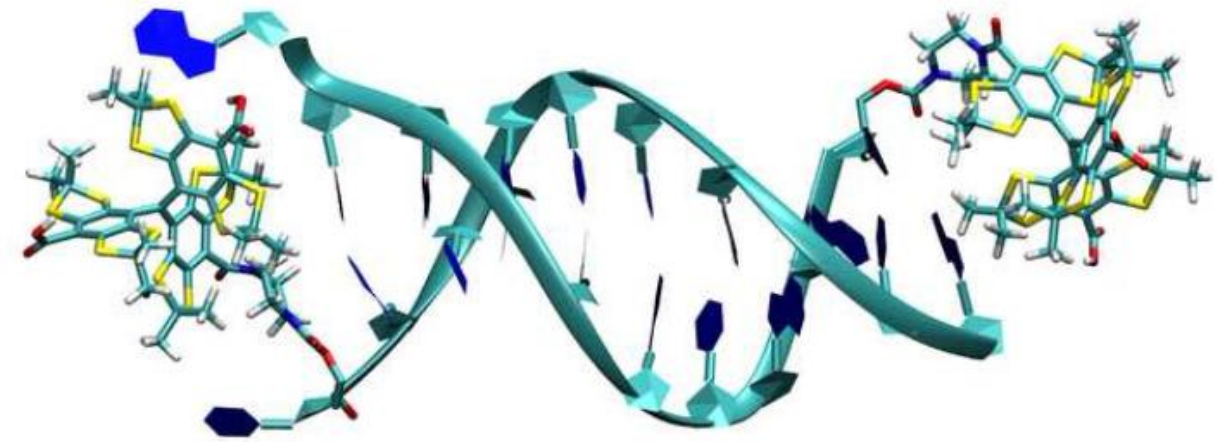
Graphical
abstract



Highlights

- DNA duplex labeled with two Triarylmethyl (TAM) spin-radicals is studied.
- 2-μs long MD simulation of aqueous solution of this labeled duplex is carried out.
- Fraying of DNA duplex is observed regardless of the presence of TAM labels.
- Inter-spin distances for unfrayed duplex states are close to EPR data on a sorbent.