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MOLECULAR DYNAMICS OF FORMATION OF TD LESIONED DNA
COMPLEXED WITH REPAIR ENZYME-ONSET OF
THE ENZYMATIC REPAIR PROCESS

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**Molecular Dynamics of Formation of TD Lesioned DNA Complexed with Repair
Enzyme - Onset of the Enzymatic Repair Process**

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To describe the first step of the enzymatic repair process (formation of complex enzyme-DNA), in which the thymine dimer (TD) part is removed from DNA, the 500 picosecond (ps) molecular dynamics (MD) simulation of TD lesioned DNA and part of repair enzyme cell (inclusive of catalytic center - Arg-22, Glu-23, Arg-26 and Thr-2) was performed. TD is UV originated lesion in DNA and T4 Endonuclease V is TD specific repair enzyme. Both molecules were located in the same simulation cell and their relative movement was examined. During the simulation the research was focused on the role of electrostatic energy in formation of complex enzyme-DNA. It is found, that during the first 100 ps of MD, the part of enzyme approaches the DNA surface at the TD lesion, interacts extensively by electrostatic and van der Waals interactions with TD part of DNA and forms complex that lasts stable for 500 ps of MD. In the beginning of MD, the positive electrostatic interaction energy between part of enzyme and TD ($\sim +10$ kcal/mol) drives enzyme towards the DNA molecule. Water-mediated hydrogen bonds between enzyme and DNA help to keep complex stable. As a reference, the MD simulation of the identical system with native DNA molecule (two native thymines (TT) instead of TD) was performed. In this system the negative electrostatic interaction energy between part of enzyme and TT (~ -11 kcal/mol), in contrary to the positive one in the system with TD, doesn't drive enzyme towards DNA and complex is not formed.

Keywords: Thymine Dimer - T4 Endonuclease V Complex, Molecular Dynamics, Recognition Process

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チミンダイマー損傷DNAと修復酵素の複合体形成の分子動力学 - 修復過程の開始

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チミンダイマー (TD) 損傷部分をDNAから除去する修復過程の第1段階 (酵素とDNAの複合体の形成) について調べるために、TD損傷DNAと修復酵素の一部分 (活性中心の Arg-22, Glu-23, Arg-26, Thr-2 を含む) に関する 500 ps の間の分子動力学シミュレーションを行った。TD は紫外線によりDNAに生成される損傷で、T4 Endonuclease V はこの損傷に特異の修復酵素である。TD とT4 Endonuclease V の一部を同じシミュレーションセルの中に配置し相対的な動きを調べた。酵素とDNAの複合体形成における静電エネルギーの役割に焦点をあててシミュレーションを行った。最初の 100 ps の間に酵素の一部がDNAのTD損傷部分に近付き、さらに静電力とファンデアワールス力を介して作用し複合体を形成し、500 ps の間安定な状態を持続した。最初の段階では正の静電作用エネルギーが酵素をDNAに向けて動かした。水分子を仲介とした水素結合が複合体を安定に保つのを助けた。比較のために、損傷のないDNAに関して同様のシミュレーションを行ったが、この場合には、負の静電作用エネルギーにより複合体は形成されなかった。

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1. Introduction

A general mechanism by which a number of sequence specific DNA-interactive proteins locate target sites within large domains of DNA has been shown in vitro to be a one-dimensional diffusion process, which is termed scanning or sliding. It has been proposed that proteins interact electrostatically with non-target DNA and then slide along DNA by a random walk [1, 2]. In DNA-bound proteins, the specific base sequences in the DNA are directly and indirectly recognized through the hydrogen bonds, the van der Waals interactions, the hydrophobic interactions, and mainly through the non-specific electrostatic interactions between negatively charged phosphate backbone of DNA and the basic residues of the protein [3]. In general, a proteins scan double stranded DNA molecules by electrostatic interactions, recognizes the site with non-conformity and sequentially bind to DNA [4]. Electrostatic forces are also able to steer the protein into a partially correct orientation for binding to DNA, an effect that in addition to the temperature dependence of binding constant, has been more generally attributed to the hydrophobic effects [5, 6]. In addition to electrostatic interactions, several proteins monitor DNA molecules also for structural aberrations which may have been caused by radiation, chemical or thermal challenge, or which may have arisen during DNA replication. One of these proteins, enzyme T4 Endonuclease V, recognizes and initiates repair of cyclobutane pyrimide dimers in UV irradiated DNA. Pyrimidine dimers are the major photoproduct induced by UV light and have been correlated with mutations and skin cancer. T4 Endonuclease V slides on non-target sequences and progressively incises at all dimers within the same duplex DNA prior to dissociating from DNA molecule. This enzyme binds only to double stranded DNA in two step process: at first it scans non-target DNA sequences by electrostatic

interactions to search for damaged sites, and sequentially specifically recognizes the dimer sites [7, 8]. The structural and energetic features of the enzyme in the absence of DNA has been described in detail in several studies [e.g. 9, 10, 11]. In addition to experimental and theoretical studies of cis.syn dimers containing DNA molecules [e.g. 12, 13, 14], a remarkable crystal structure of the enzyme bound to DNA with cis.syn thymine dimer (TD) has been reported [15]. Availability of crystallographic coordinates allows to use the molecular modeling techniques to obtain an insight view into the dynamics of important molecular processes leading to deeper understanding how these photodamaged duplexes are recognized by repair enzyme. The recent theoretical study revealed that the electrostatic energy of thymine dimer site on DNA molecule has significant negative value in contrary to nearly neutral value of the native thymine site [16]. Specific electrostatic energy of damaged site might be detected by enzyme during scanning process. This result, in addition to the above mentioned experimental and theoretical observations showing the importance of electrostatic energy in recognition process, led to the simulation of TD lesioned DNA complexed with repair enzyme T4 Endonuclease V. To investigate the role of electrostatic energy in orienting and driving repair enzyme towards the DNA, and in proper binding to the DNA, the 500 picosecond (ps) molecular dynamics (MD) simulations of the two systems were performed and results are discussed in this paper. Each simulated system consists of two solute molecules solvated in water. One system consists of DNA dodecamer in which two thymines in positions 6 and 7 are replaced with TD and part of repair enzyme, in explicit solvent water at an ambient temperature and pressure. Second system composes of the native DNA dodecamer of the same sequence and the same part of repair enzyme as in the first system. In addition to description of the role of electrostatic energy, these simulations provide an insight view into the dynamics of

recognition of the TD damage by repair enzyme and formation of a specific DNA-enzyme complex. The formation of this complex is the key step for the onset of the enzymatic repair process.

2. Materials and methods

Prior the formation of systems, the 1 nanosecond (ns) MD simulations of three solute molecules, the native DNA, DNA containing TD and T4 Endonuclease V, were performed individually for each of them. These simulations are reported in paper of Pinak [16]. Examining the positional root mean square deviations (r.m.s.d.) of individual molecules, it was found, that after 800 ps of MD each molecule reached its stable structure (Fig. 1). Positional r.m.s.d. was calculated as a deviation from the original position in the beginning of MD simulation. Structures produced at 840 ps of MD were selected as the initial molecular structures for construction of the systems. Before construction, the original velocities of each atoms were nullified, i.e. the individual solute molecules were considered as crystal structures at 0 K temperature.

Forming the system of two solute molecules (DNA and enzyme) solvated in water, a reasonable compromise between different requirements has to be found: on one hand, the dimensions of the system must be kept reasonable small to be able to proceed with MD simulations, and on the other hand system should be big enough to obtain general features of the recognition process. Formation of system consisting of DNA dodecamer and whole enzyme T4 Endonuclease V would result in a big system composing of nearly 40,000 - 50,000 atoms in total (including atoms of solvent water). Considering these requirements and available computer equipment, finally the system of the 2 solute molecules was chosen - DNA dodecamer as one molecule and one part of enzyme as a second molecule.

Amino acids composing the part of enzyme in the simulated system were selected considering the structural as well energy properties of the whole enzyme molecule. T4 Endonuclease V consists of three α helices (H1 - amino acids 14 through 38, H2 - 64 through 82 and H3 - 108 through 124) standing side by side, several reverse turns and several loops [17]. Glu-23, of which carboxyl chain plays a crucial role in the cleavage of N-glycosyl bond in DNA during enzymatic repair process [18,19], is surrounded by amino acids Arg-3, Arg-22 and Arg-26 belonging to helix H1. Side chain of Glu-23 also forms a hydrogen bond with the backbone amino group of Arg-3. Arg-3 and Thr-2 - two polar residues at the NH_2 terminus - form several hydrogen bonds with the side chains of helices H1 and H2, and lie on the molecular surface. Considering these properties, the 8 amino acids of H1 - Glu-20, Tyr-21, Arg-22, Glu-23, Leu-24, Pro-25, Arg-26, Val-27, and 2 amino acids at NH_2 terminus - Thr-2 and Arg-3 were selected to form the simulated part of enzyme (Fig. 2). Amino acids - Thr-2, Arg-22, Glu-23 and Arg-26, form so called catalytic center that is active in the incision of thymine dimer during repair process, and together with other 6 selected amino acids - Arg-3, Glu-20, Tyr-21, Leu-24, Pro-25 and Val-27, lie at the central part of concave site of the enzyme. This part of enzyme may be easily exposed to the DNA surface and has been shown as having possible contacts with TD atoms of DNA in crystal structure of DNA complexed with T4 Endonuclease V [15]. Extensive hydrogen bond network between amino acids of helix H1 and Thr-2 and Arg-3 at the surface shall ensure stability of selected part of enzyme during MD simulation. In addition to structural criteria, amino acids were selected to represent typical electrostatic energy of those composing whole enzyme molecule (in average +10 kcal/mol [16]).

Molecular dynamics simulations were performed with the program AMBER 5.0 [20] using the standard all-atom force field parameters for nucleic acids and protein amino

acids as included in the program. This program was used for simulation of two systems. One system composed of the lesioned DNA dodecamer of the sequence $d(\text{TCGCG}'\text{TD}'\text{GCGCT})_2$ and part of the enzyme T4 Endonuclease V as specified above. TD in DNA sequence represents thymine dimer formed as a complex of two thymine bases at positions 6 and 7 joined by C(5)-C(5) and C(6)-C(6) bonds of which structure was optimized during minimization process (Fig. 3). The atomic charges of TD were used as those calculated by Miaskiewicz et al. [12]. The second system composed of the native DNA dodecamer of the same sequence as lesioned one, with two thymines TT instead of TD, and the identical part of the enzyme as it is in the first system.

Formation of DNA-enzyme systems - Simulated Annealing

1. Using a molecular graphic software InsightII [21] the part of enzyme was placed close to DNA dodecamer (native or with TD), and oriented such way that its catalytic center faced to the TT (TD) site on dodecamer (Figs. 4a) and 4b)). The relative distance of DNA and part of enzyme was chosen as to minimize mutual van der Waals interactions between the respective molecules. This way two systems were formed - native DNA + part of enzyme and, DNA with TD + identical part of enzyme. After the formation of systems the solute molecules were solvated in water environment. The neutrality of each system was achieved by adding sodium counterions Na^+ at the initial positions bisecting the P-O-P angle at the distance 5 Å from each phosphorus atom of DNA which are having negative charges. During the MD simulation no further restraint on the position of sodium atoms was applied. Each system finally composed of nearly 20,000 atoms in total.

Constructed systems were subjected of simulated annealing (SA) in duration of 15 ps:

- 1 ps, heating up to 1,200 K from initial 0 K;
- 2 ps, keeping at 1,200 K;
- 12 ps, cooling to 0 K in 3 stages with different coupling constants.

During simulated annealing the all nucleotides of DNA dodecamer were restrained in Cartesian space using a harmonic potential with harmonic force constant of 1 kcal/mol. The reason for constraining DNA atoms was high annealing temperature of 1,200K, that might easily result in breaking of hydrogen bonds between corresponding nucleotides of DNA strands. No restrain was applied on the amino acids of the enzyme.

When the SA finished, the MD simulations were performed for each system. Details of the MD protocol have been reported elsewhere [e.g. 13, 16, 22]. In native system as well in system with TD, the MD protocol composed of minimization of potential energy, heating up to 300 K from 0 K at the end of SA, stabilization of density of the system and finally production MD with periodic boundary conditions. In Fig. 5, the temperature profile of the system during preparation steps can be seen.

Simulations were performed on FUJITSU VPP500/42 vector/parallel type supercomputer of the Japan Atomic Energy Research Institute (JAERI), Tokai Research Establishment. In original code AMBER 5.0 the sequential and parallel flags were changed to compile program on the VPP500/42 computer. One ps of MD simulation of constructed systems required approximately 1.7 hours of CPU time.

Force Field and Calculation of Electrostatic Energy

Atomic charges, van der Waals, hydrogen bonds, angles and other atomic parameters were taken from AMBER 5.0 all-atom force field [20]. Potential energy function in AMBER 5.0 is calculated as contribution from bond, single angle, torsional, improper dihedral, van der Waals, hydrogen bonding, electrostatic and constraint functions:

$$\begin{aligned}
E_{potential} = & \sum_{bonds} K_r (r - r_{eq})^2 + \sum_{angles} K_\theta (\theta - \theta_{eq})^2 + \\
& \sum_{dihedrals} \sum_{\eta} \frac{V_\eta}{2} [1 + \cos(\eta\phi - \gamma)] + \\
& \frac{1}{VDW_{scale}} \sum_{j=1}^{atoms} \sum_{i>j}^{atoms} \epsilon_{ij}^* \left[\left(\frac{R_{ij}^*}{r_{ij}} \right)^{12} - \left(\frac{R_{ij}^*}{r_{ij}} \right)^6 \right] + \\
& \sum_{j=1}^{Hbonds} \sum_{i>j}^{Hbonds} \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} \right) + \\
& \frac{1}{EEL_{scale}} \sum_{j=1}^{atoms} \sum_{i>j}^{atoms} \frac{q_i q_j}{\epsilon r_{ij}} + \\
& \sum_{constr.} K_{constr} (x - x_o)^2 + \sum_{cap\ atoms} K_{cap} (y - y_o)^2
\end{aligned}$$

Here r , θ and ϕ are bond lengths, planar angles and dihedral angles respectively; r_{eq} and ϕ_{eq} are their equilibrium values; r_{ij} is the distance between atoms i and j ; q_i is the partial charge on atom i ; ϵ_{ij} is dielectric constant; K_{constr} , K_{cap} , K_θ , V_η , R_{ij} , C_{ij} and D_{ij} are empirical parameters depending on atom types. The point charges q_i and q_j in calculation of electrostatic function (sixth term in Eq. 1) are centered on each atom and are derived by fitting to quantum mechanical electrostatic potential. In MD simulation the constant dielectric function was used, 1-4 electrostatic interactions were scaled by factor 1.2, that is recommended value for Amber 5.0 force field, and Particle Mesh Ewald Sum technique was used as implemented in Amber 5.0 [23]. Using Ewald Sum method the electrostatic energy was calculated for infinite volume of repeating units through periodic boundary conditions. This method is computational technique for calculating long range electrostatic interactions which are often neglected while using a finite non-bonded cutoff in MD simulations. In the Ewald sum method, a Gaussian charge distribution of opposite sign is superimposed upon the original; point charges, producing a screened charge distribution. The electrostatic interaction between the screened charges are short ranged. The original distribution is recovered by adding a

second Gaussian charge distribution identical to the first, but of opposite sign. The interaction between these canceling distributions is calculated in reciprocal space. The total electrostatic interaction in the entire simulated volume is then calculated as:

$$E_{total} = E_{real} + E_{reciprocal} - E_{self} - E_{excl} \quad , \text{ where} \quad \text{Eq. 2}$$

$E_{(real)}$ is the screened interaction and $E_{(reciprocal)}$ is due to the canceling Gaussian charge distribution. $E_{(self)}$ corrects for the interactions of the canceling charge distribution between an atom and all excluded atoms - E_{excl} (i.e. atoms that are linked through a bond, angle or torsion to an atom).

3. Results and discussion

3.1 Structural analysis

In order to investigate the dynamics of the systems, the r.m.s.d. of each solute molecule was calculated. Analysis of its trajectory may point on the significant structural changes that are undergoing in the respective molecule. Calculated r.m.s.d. represents an average deviation over the all atoms of solute molecule from their original positions at the formation of system, i.e. prior the simulated annealing. R.m.s.d. of part of enzyme and native DNA molecule in the system show large fluctuations during the approximately first 300 ps indicating movement of molecules as well the structural changes of entire molecules. After 300 ps the r.m.s.d. of both molecules stabilized and remained stable up to performed 500 ps of MD simulation (Fig.6a)). In the case of system with TD, the r.m.s.d. of both molecules rise sharply at the beginning of the simulation, stabilize after 100 ps, and remain stable throughout the all MD simulation (Fig. 6b)). In both systems, with as well without TD, the main

positional and structural changes that are reflected in r.m.s.d. occurred in the beginning of MD, i.e. during SA, consequent heating and density stabilization runs (first 31 ps).

The both systems were formed to be initially identical in relative positions of both solute molecules - DNA and part of enzyme (see Figs. 4a) and 4b)). Fig. 7a) shows snapshots of both molecules in the system with native DNA at various moments during simulation. It can be seen that the part of enzyme moves slightly further from DNA and then both solute molecules keep stable at their positions. Examining their r.m.s.d., solute molecules reached stable position and structure after 300 ps of MD. Fig. 7b) shows snapshots of solute molecules in system with TD taken at the same moments as those shown in Fig. 7a). It can be seen that the part of enzyme moved in direction to TD site during SA and formed the possible contacts between the respective atoms of enzyme and backbone of DNA. Comparing the respective r.m.s.d. of these two molecules it is found that this movement lasted during first 100 ps and the formed complex part of enzyme-DNA kept stable afterwards.

Looking at possible contact area between DNA with TD and enzyme on Fig. 8, it can be seen, that there is an intensive interaction arising from the close vicinity of Arg-22 and Tyr-21 to C3', and Arg-26 to C5' of TD site. Arg-26 comes very close to the C5' of DNA backbone and occupies the space near the Gua-5 and TD. This specific position of Arg-26 helps to define the correct orientation of enzyme with respect to TD. It shall be noted, that Arg-22 and Arg-26 contribute significantly to the recognition and ensure the stability of the complex structure. They are also involved in the interactions with TD. Glu-23, cleaving the N-glycosyl bond in DNA during repair process, approaches the C5' atom of DNA backbone (distance ~5 Å). These observations are in good agreement with crystal structure of DNA and enzyme, where side chains of Arg-22 and Arg-26 form stacking contact with DNA [15].

The formed complex of enzyme and DNA lasted stable throughout the simulation after 100 ps which also suggests the existence of extensive hydrogen bonds interaction between them. Close look at the area between DNA and part of enzyme shows that there are located several water molecules (Fig. 9). These water molecules occupy “empty” space between two solute molecules and are at distance 5 Å or less to the closest atom in either solute molecule. These water molecules are probably involved in water-mediated hydrogen bonds and help to keep the complex stable. The stability of complex is necessary for the onset of the catalytic process in which the glycosyl bond at C5' side and phosphodiester bond at the C3' of TD are incised [e.g. 8, 24].

3.2 Role of electrostatic energy in recognition process

As described in *Materials and Methods*, the potential energy in simulated system is calculated as contributions from bonds, angle, van der Waals, electrostatic and various constraint energy functions. Further the Particle Mesh Ewald sum with no cut-off distance is applied for calculation of electrostatic energy, that ensures the calculated values represent the electrostatic interaction between the all atoms of respective DNA nucleotide as well part of enzyme. Atomic parameters for calculation of individual terms of potential energy are taken from AMBER 5.0 all-atom force field with necessary modifications for TD [12, 19]. The above described structural changes, mainly the approach of part of enzyme to DNA, recognition of TD site and formation of complex are originated by the energy conditions in the system during MD. As mentioned in *Introduction*, it is suggested by experimental and theoretical works, that electrostatic energy is the main factor helping the proper recognition of TD site on DNA [e.g. 4, 16]. In order to examine the role of electrostatic energy in the formation of complex, this was calculated throughout the simulation. Because the performed

simulation represents simulation of only one part of enzyme molecule, the electrostatic interaction energy was calculated between the part of enzyme and TD, and between part of enzyme and TD neighboring nucleotides (Gua-5 and Gua-8). Fig. 10 shows the interaction energy throughout the simulation. It is seen that in the formation of the system, i.e. prior the SA, the interaction energy between TD and enzyme was positive of around +10 kcal/mol. This positive value has origin in attractive interaction between negative electrostatic energy of TD and positive electrostatic energy of part of enzyme. This attractive force drove the enzyme towards the TD site on DNA. This would be in coincidence with experimental suggestions, that the damaged site is recognized during electrostatic scanning of DNA surface [4]. After enzyme approached the DNA surface, the energy became negative, which was mainly originated by two arginines (Arg-22 and Arg-26) which both are having highly negative electrostatic energy of around -50 kcal/mol [16], and are located at the close positions to DNA. This, in combination with negative charge of DNA phosphate backbone creates repulsive environment, in which the enzyme is already docked into the DNA. It is highly probable, that after the enzymatic cleavage process cuts the TD part out of DNA, this repulsive force could break the hydrogen bonds that are connecting enzyme and DNA. Result of broken hydrogen bonds is that the enzyme then dissociates from DNA molecule. As can be seen on Fig. 10, the major repulsive interaction is between enzyme part and TD part if compared with repulsive interaction between enzyme part and neighboring guanines (Gua-5 and Gua-8).

Because both solute molecules are very close to each other, the van der Waals energy is another factor that may contribute to the stability of formed complex. Examining the van der Waals energy, it is found that it slightly resembles the electrostatic interaction

energy, being slightly attractive in the beginning of simulation and becoming repulsive after the complex is formed (Fig. 11).

Referring to the interactions between repair enzyme and DNA found in crystallographic complex [15], it is seen that in crystal complex these are mostly electrostatic and polar interactions of enzyme atoms with atoms of DNA nucleotides forming TD. The electrostatic interaction is also considered as restraining the orientation of DNA strand in respect to enzyme. In addition there is a water-mediated hydrogen bonds network between the enzyme and DNA, that is also observed in this MD simulation (see Fig. 9).

Calculating the electrostatic interaction energy between solute molecules in the system with native DNA, it is found that in the beginning of the simulation, the energy is negative of around -11 kcal/mol, in contrary to positive energy (+10 kcal/mol) in the case of system with TD. Native thymines have an electrostatic energy of nearly neutral value and don't contribute to creation of the attractive environment needed for approaching of enzyme to DNA as observed in the system with TD. Repulsive force drives the part of enzyme further from DNA as can be seen in the relative positions of these two solute molecules (see Fig. 7a). This repulsive force between phosphate backbone of DNA with neutral thymine part 'TT' and negative arginines in relatively small simulated part of enzyme became dominant. After the solute molecules were at positions with bigger distance from each other, the electrostatic interaction energy between DNA and part of enzyme became neutral (~0 kcal/mol), and solute molecules stabilized at the of distance ~15 Å.

Other factors contributing to total potential energy of the system, e.g. bonds, angles, constraint energy functions and atomic parameters, were also examined but proved to be similar in both systems and stable throughout the MD (graphs not shown).

4. Conclusion

The MD simulations of two systems, each of them containing two solute molecules - DNA (native or with TD) and part of specific repair enzyme T4 Endonuclease V are presented. The results confirm the idea, that the electrostatic energy is one of the significant factors in recognition of TD damaged site on DNA molecule. Specific negative value of electrostatic energy of TD site, different from nearly neutral of native non-damaged TT site, helps in orienting and driving of repair enzyme towards the TD on DNA. In simulation of system with native DNA, no significant movement of neither part of enzyme nor DNA was observed. In simulation of system containing TD damaged DNA, the simulated part of repair enzyme approached the DNA surface at the TD region, attached to the DNA and formed complex enzyme-DNA lasted stable up to 500 ps of performed MD simulation. As in performed simulations only relatively small part of enzyme is used for the formation of both systems, there is arising limitation in description of formation of DNA-enzyme complex and its stability. Therefore the examination of results was focused mainly on the description of structural properties at the contact area between simulated part of enzyme and DNA, and on the possible role of electrostatic energy in the recognition process. Examining the contact area, the Glu-23 is found located at close position to C5' atom of TD (~5 Å) and the Arg-26 and Arg-22 in close vicinity to C5' and C3' atoms of DNA backbone, respectively. These two atoms are being cleaved during the repair process. Docking of Arg-26 into the space between Gua-5 and TD of DNA formed the very stable complex structure. Presence of several water molecules in between both molecules in complex suggests an idea, that water-mediated hydrogen bonds are

helping to create stable environment needed for the onset of the enzymatic process and it might be the subject of future research. Formed complex might be also useful for theoretical study and dynamics simulations of the enzymatic repair process.

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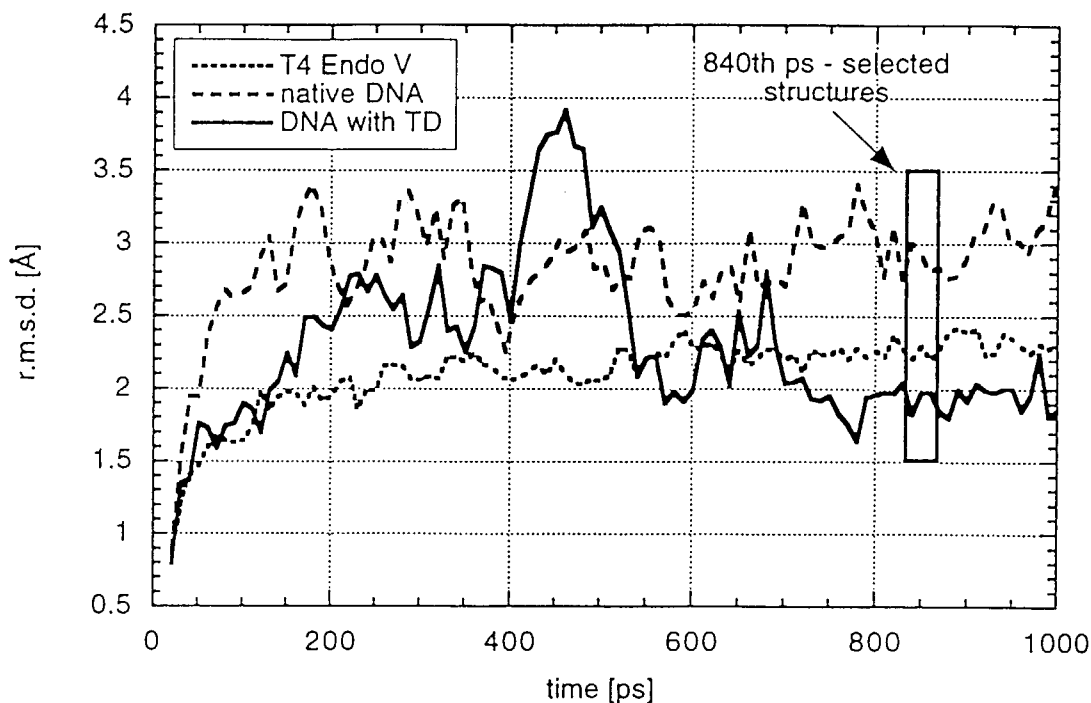


Fig. 1

R.m.s.d. of T4 Endonuclease V, native DNA dodecamer and DNA dodecamer with TD of the same sequence as native one. R.m.s.d. is calculated as positional deviations of all solute atoms (except hydrogens) from their original positions in the beginning of the MD simulation. In addition to several stable intervals of various lengths during the first 800 ps, all molecules reached stable r.m.s.d. afterwards. The all MD simulation were performed individually for single solute molecule solvated in water environment [16].

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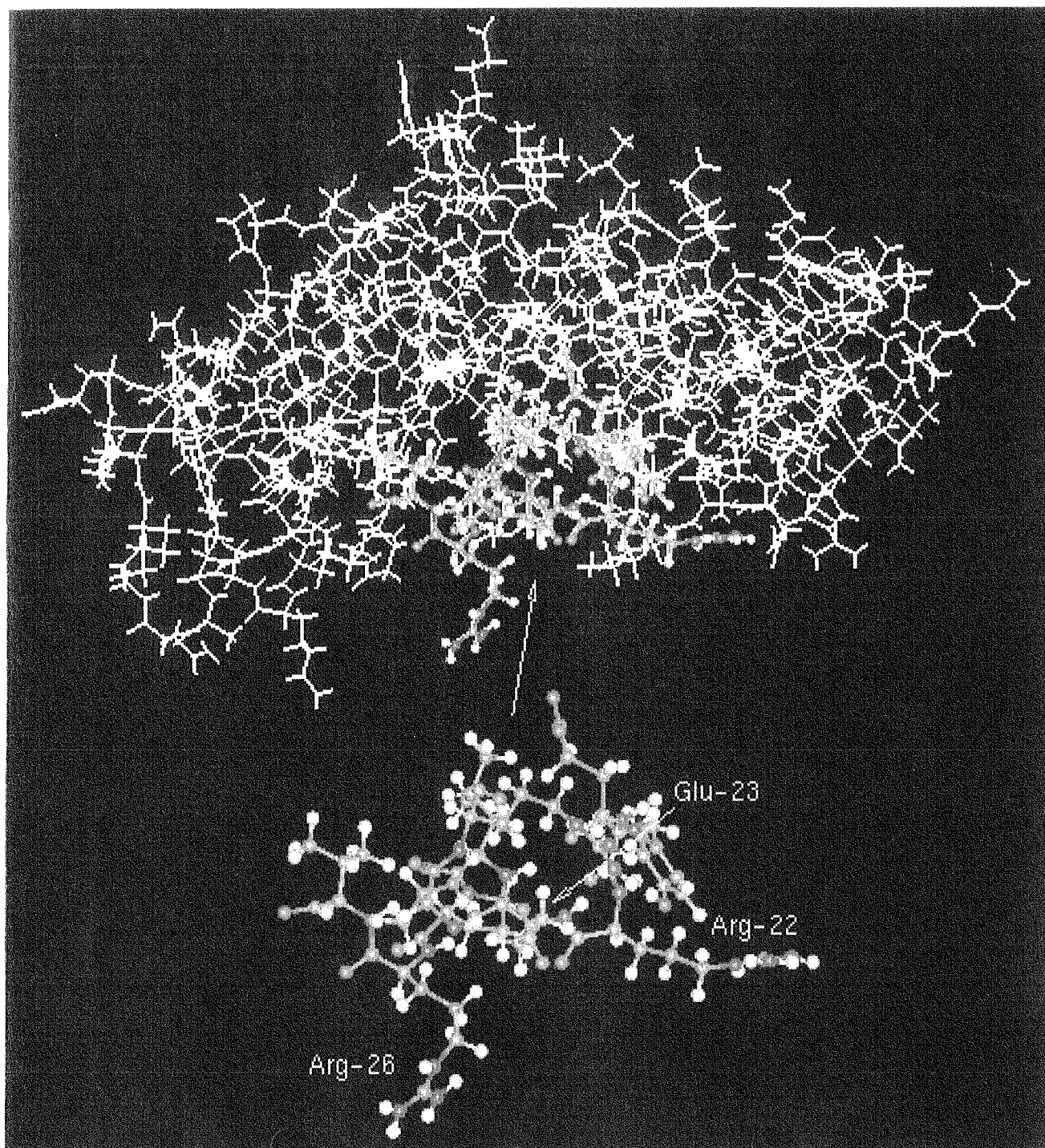


Fig. 2

Crystal structure (temperature 0 K) of T4 Endonuclease V at the 840th ps of MD simulation. The colored and enlarged are the four amino acids of catalytic center - Arg-22, Glu-23, Arg-26, Thr-2, and the six amino acids surrounding it - Arg-3, Glu-20, Tyr-21, Leu-24, Pro-25, Val-27. These 10 amino acids form the part of enzyme that is used in MD simulations.

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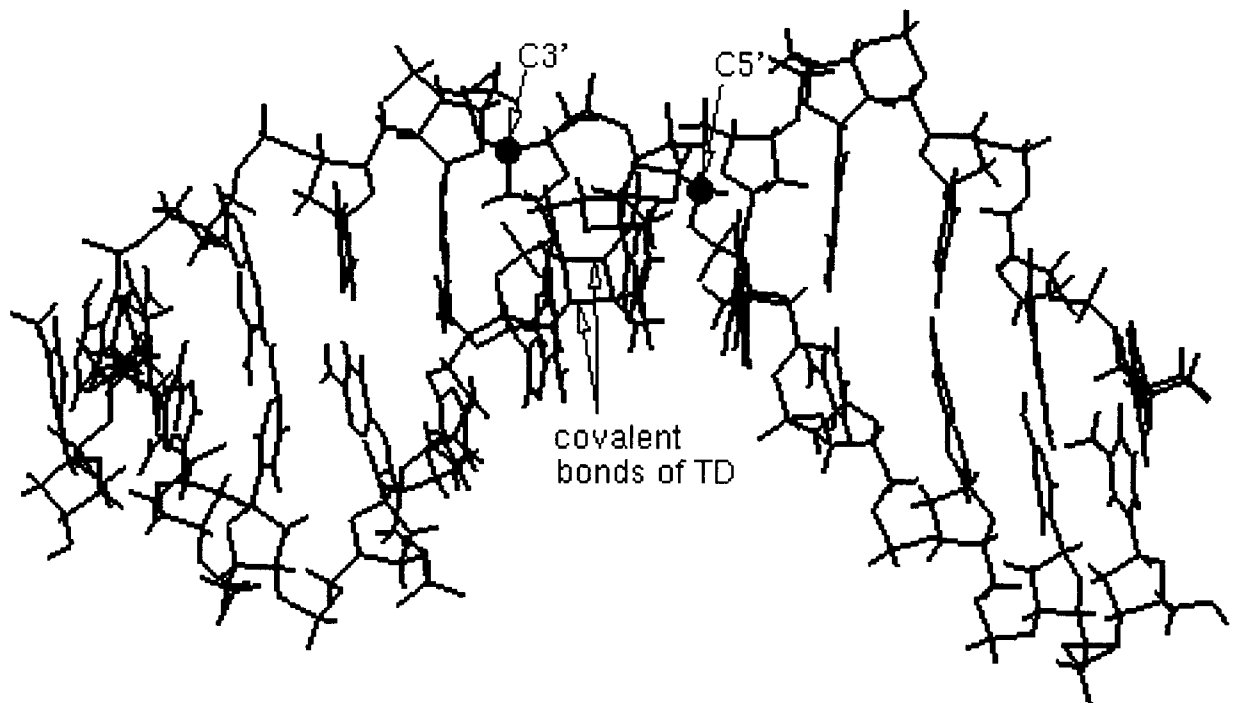


Fig. 3

Crystal structure (temperature 0 K) of DNA dodecamer $d(\text{TCGCG}'\text{TD}'\text{GCGCT})_2$. TD represent thymine dimer formed by joining of two thymines by covalent bonds. Bonds at C5' and C3' side of thymine dimer are being cleaved during the catalytic repair process. Structure taken at 840th ps of MD simulation.

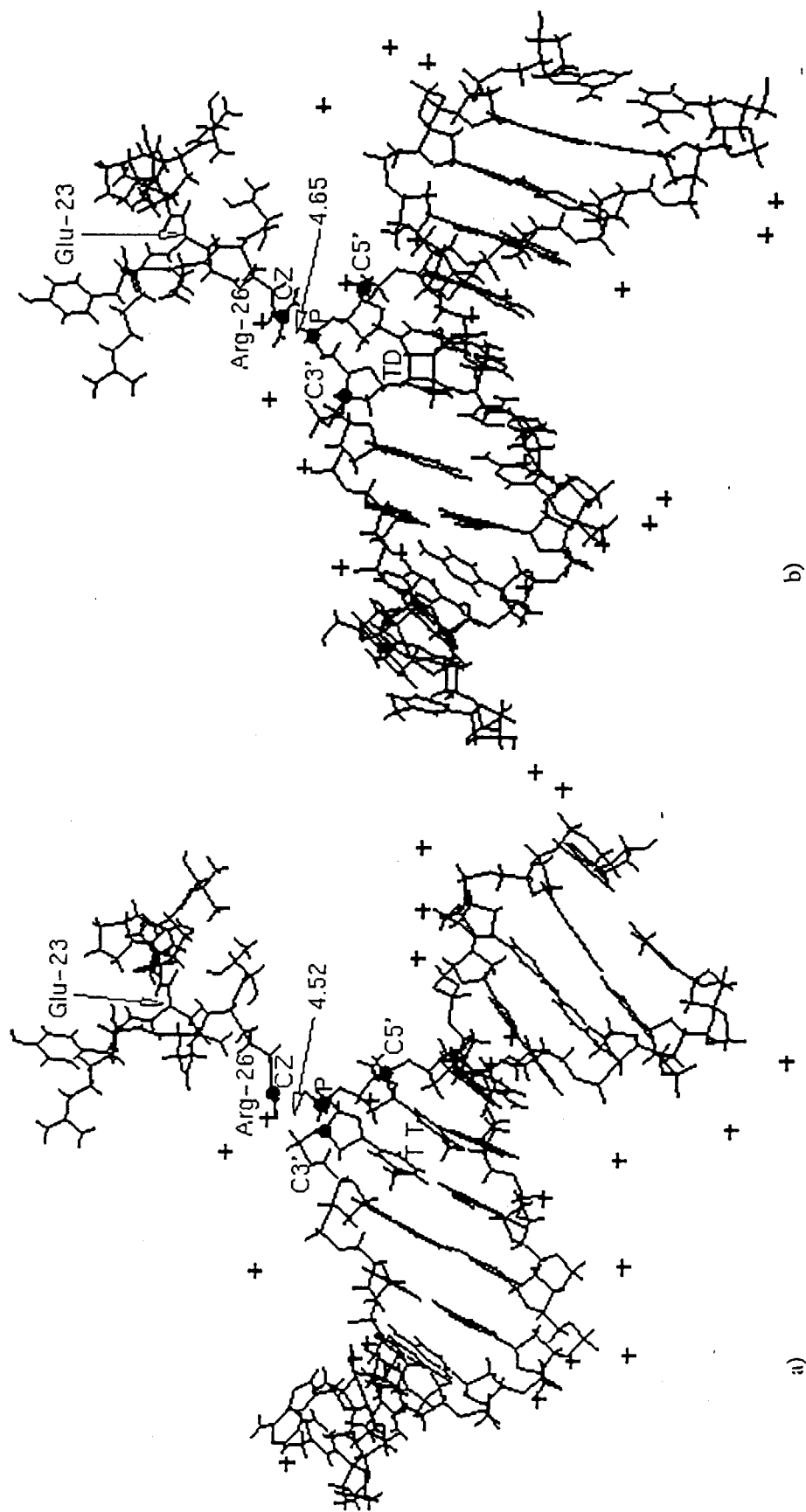


Fig. 4
 Original position of simulated part of enzyme (colored atoms on Fig. 2) and DNA dodecamer. Numbers indicate the distance in Angstroms between the closest atoms (heavy atoms, not hydrogens) of respective solute molecules (CZ of the Arg-26 and P of the TT (TD)). The single atoms surrounding DNA molecule displayed by cross marks are initial positions of sodium counterions neutralizing the negative charges of DNA phosphates.

Fig. 4a) system with native DNA

Fig. 4b) system with TD

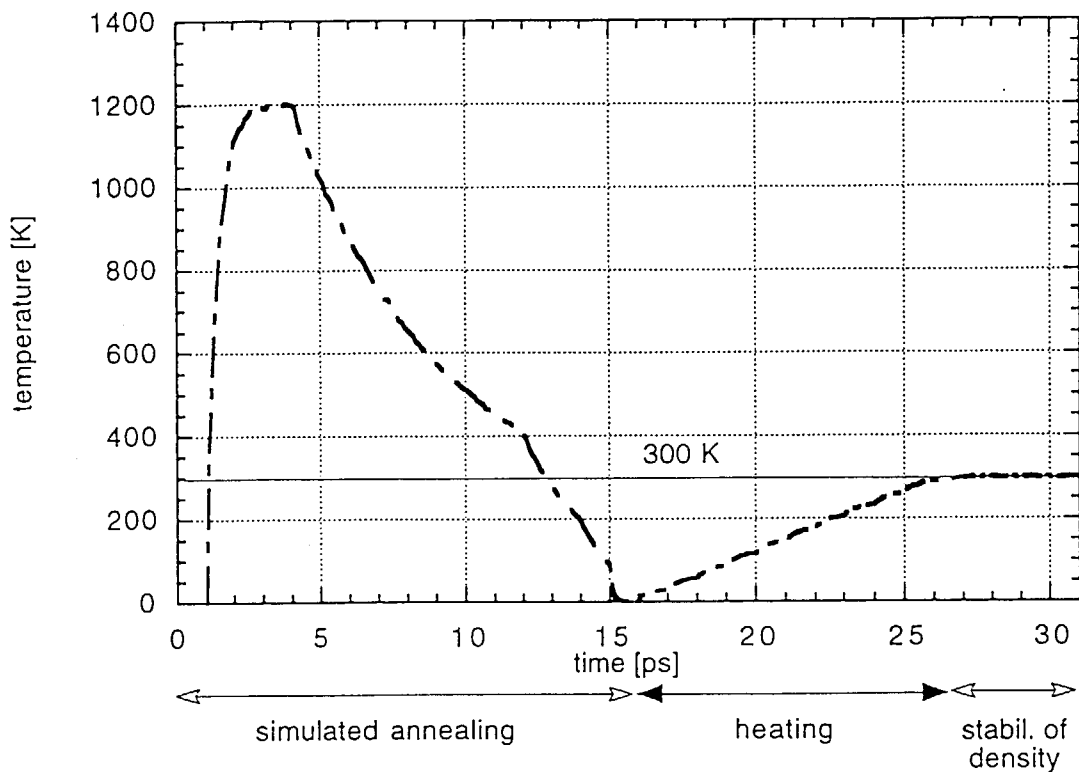


Fig. 5

Temperature profile during preparatory steps - simulated annealing, heating and stabilization of the density of the system. The atoms of DNA were kept restrained at the Cartesian space using a harmonic potential to avoid disrupting of hydrogen bond during simulated annealing.

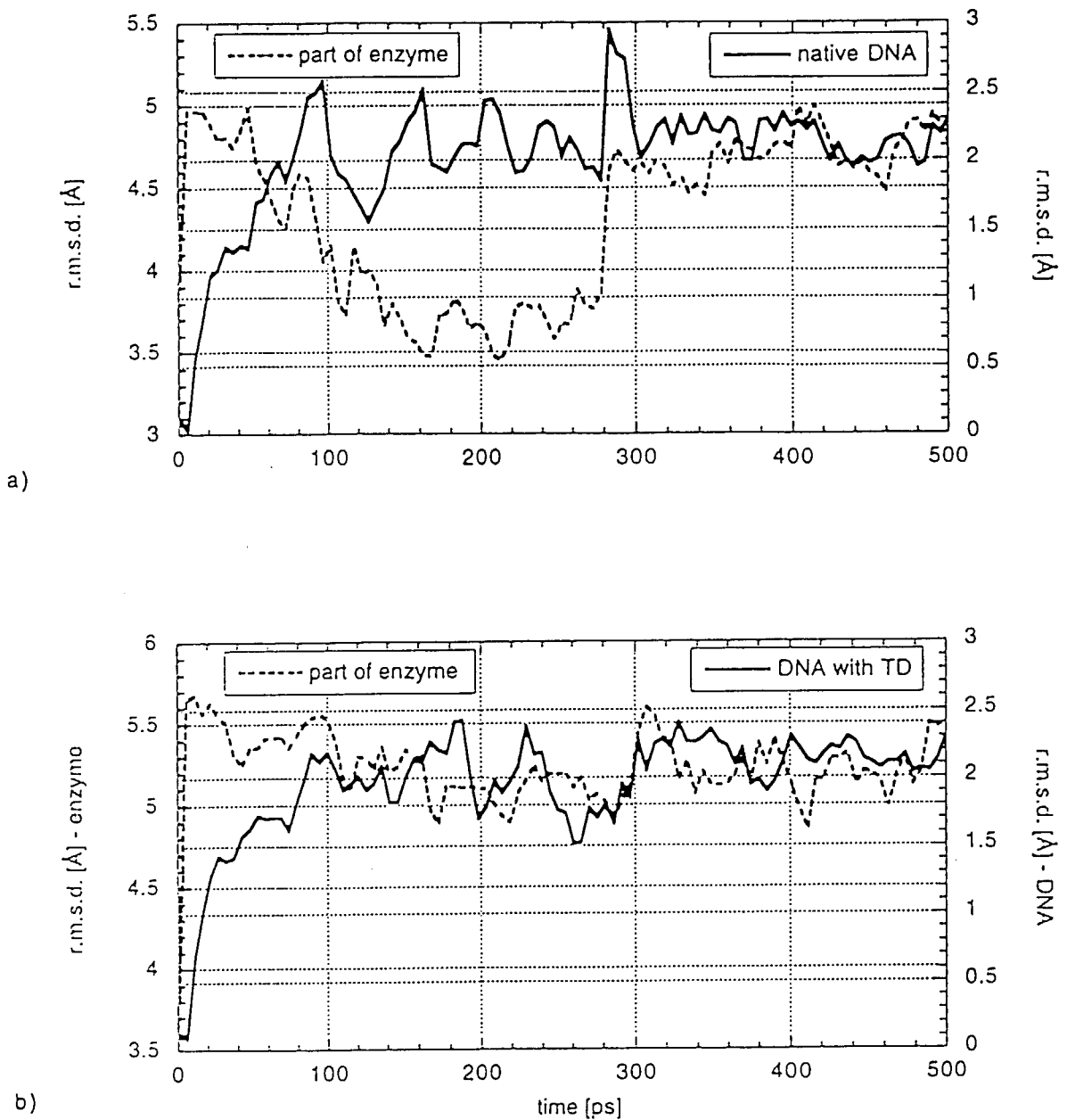


Fig. 6a)

R.m.s.d. of solute molecules in the system with native DNA. The both molecules were moving intensively during the first 300 ps, searching their stable position in the system, and stabilizing afterwards.

Fig. 6b)

R.m.s.d. of solute molecules in the system with TD. R.m.s.d. of both molecules rise sharply during the beginning of MD and keep stable oscillating around respective average values after 100 ps.

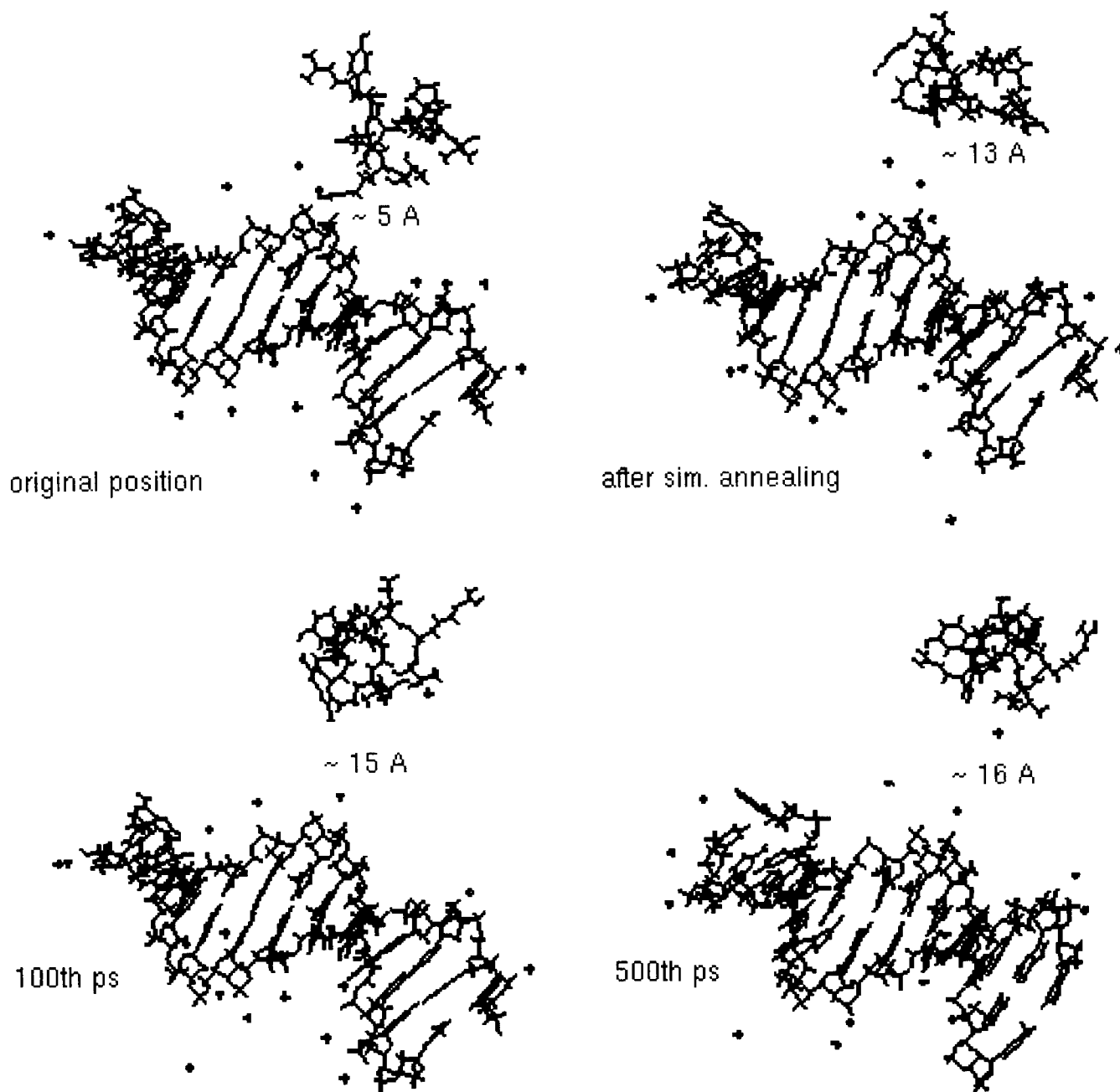


Fig. 7

Snapshots of both solute molecules taken throughout the MD simulation.

Fig 7a) system with native DNA; both molecules are moving independently from each other, increasing their original relative distance from ~5 A up to ~15 A at 300 ps and keep stable afterwards.

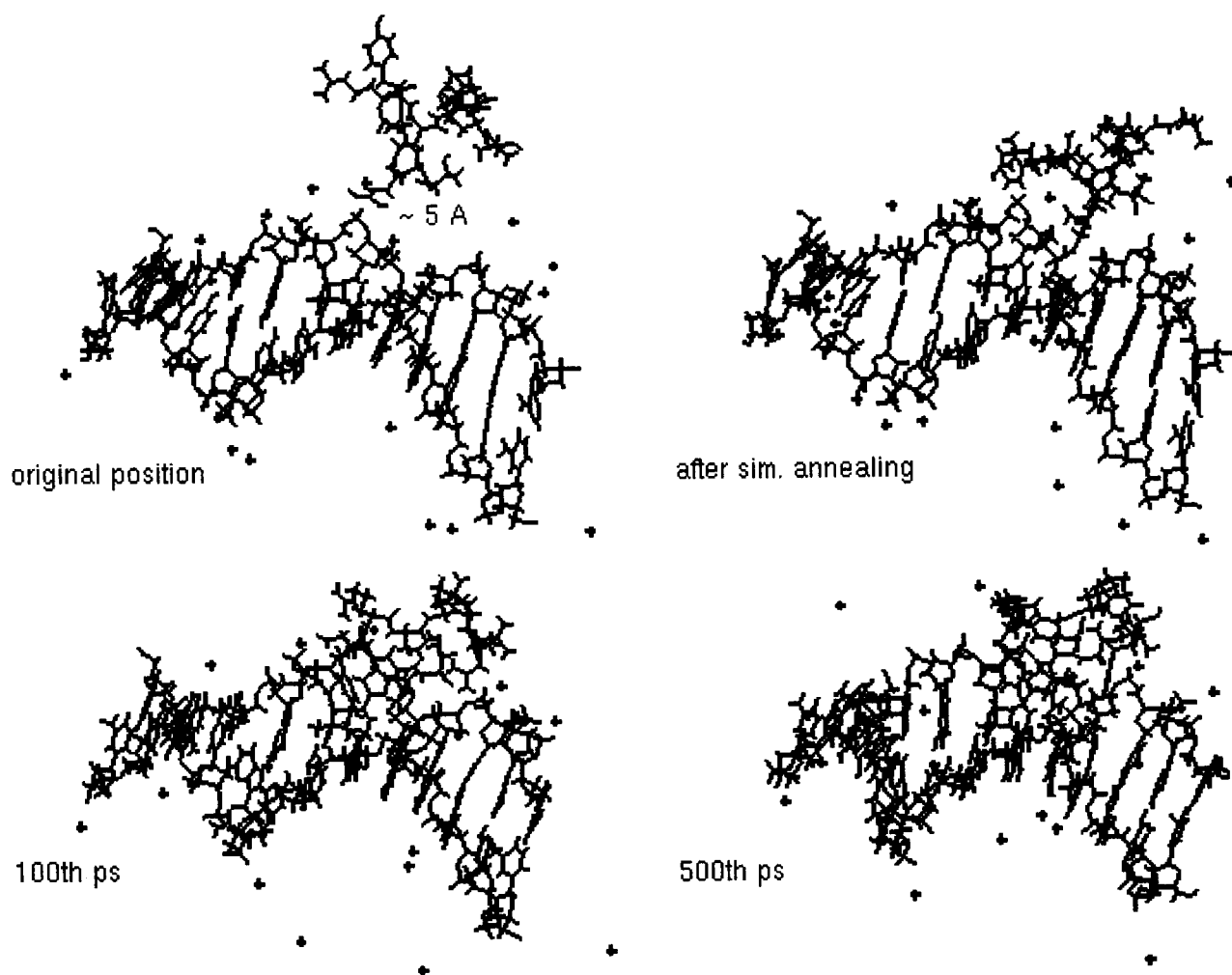


Fig. 7

Snapshots of both solute molecules taken throughout the MD simulation.

Fig. 7b) system with TD; part of enzyme approaches TD in the beginning of simulation (SA) and Arg-26 is docked between Gua-5 and TD. Complex part of enzyme-DNA keeps stable after 100 ps.

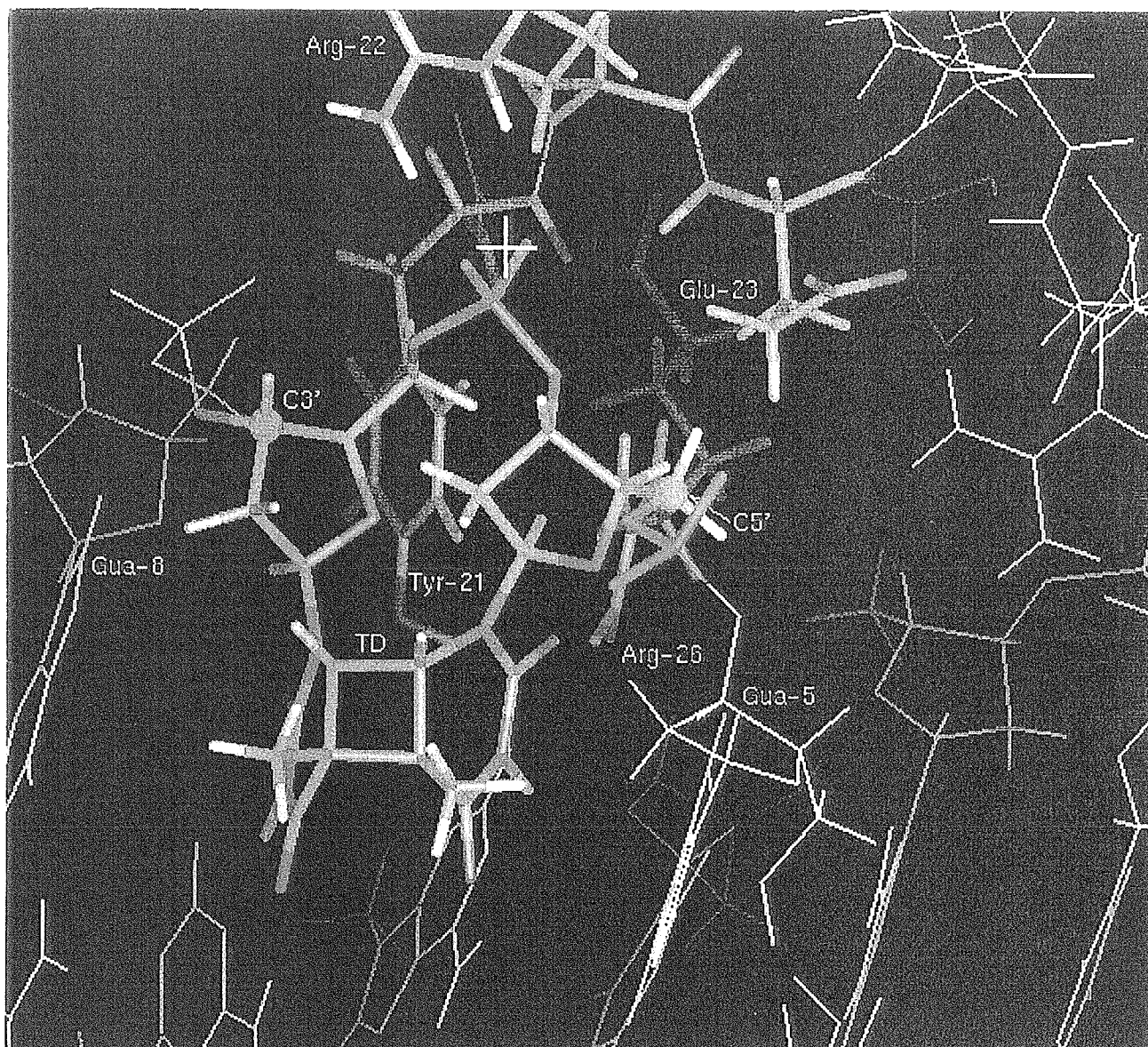


Fig. 8

Insight view into the contact area of enzyme and DNA at 100th ps of MD. Arg-26 occupies the position close to Gua-5 and TD of DNA, helping to keep the structure of complex stable. Arg-22 and Tyr-21 are closely located to the phosphodiester bond between two thymines forming TD. Glu-23, amino acid actively involved in catalytic process lies in close vicinity to the C5' atom. This configuration is kept stable throughout the performed simulation of 500 ps. (atoms are displayed using depth cueing)

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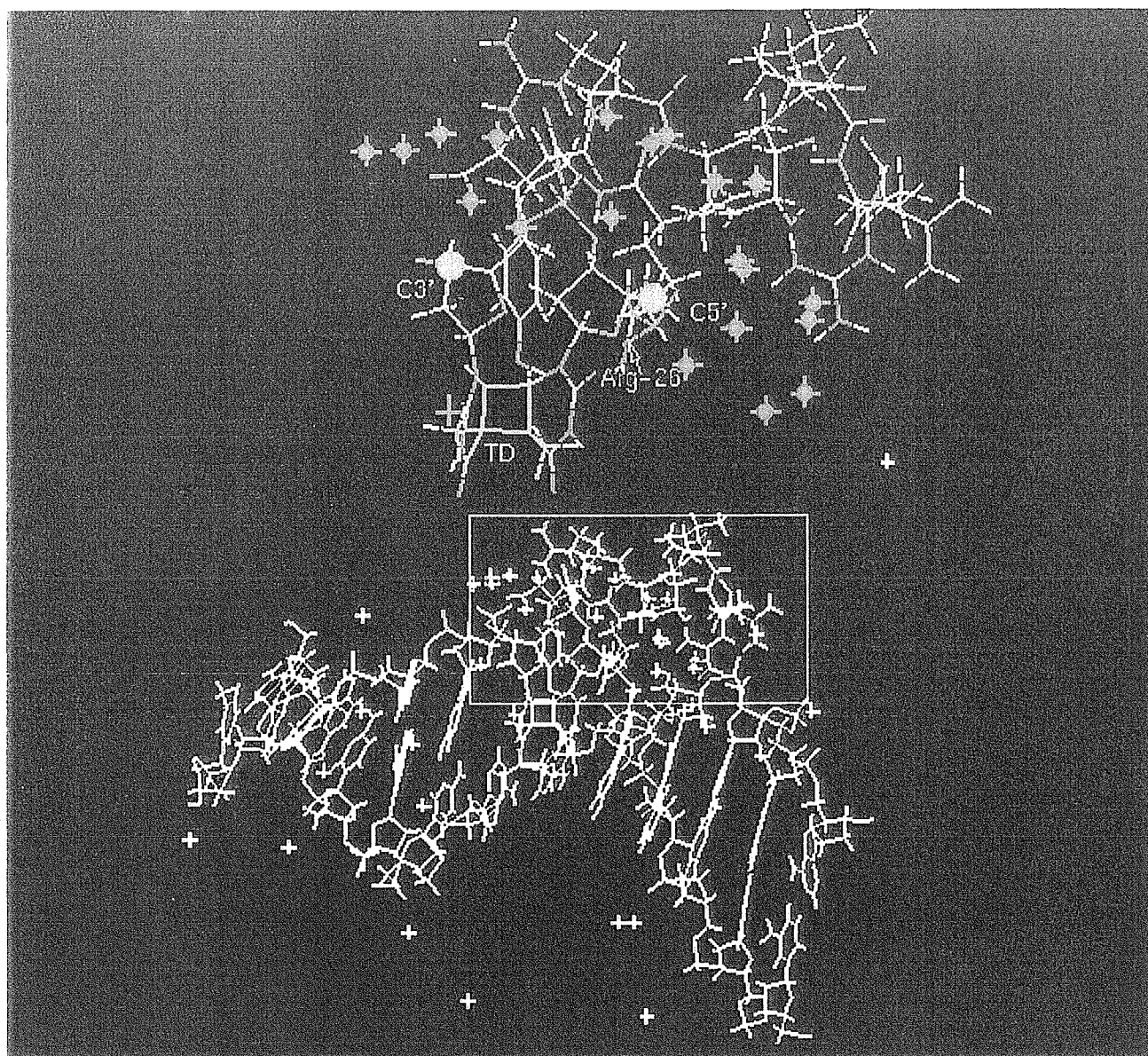


Fig. 9

Water molecules in the area between enzyme and DNA (situation at 100th ps of MD). There are several water molecules forming the possible hydrogen bonds and keeping the complex stable. Red circles represent the water oxygens. Shown are only those oxygens of water molecules of which distance to any atom of either DNA or enzyme is equal or less than 5 Å.

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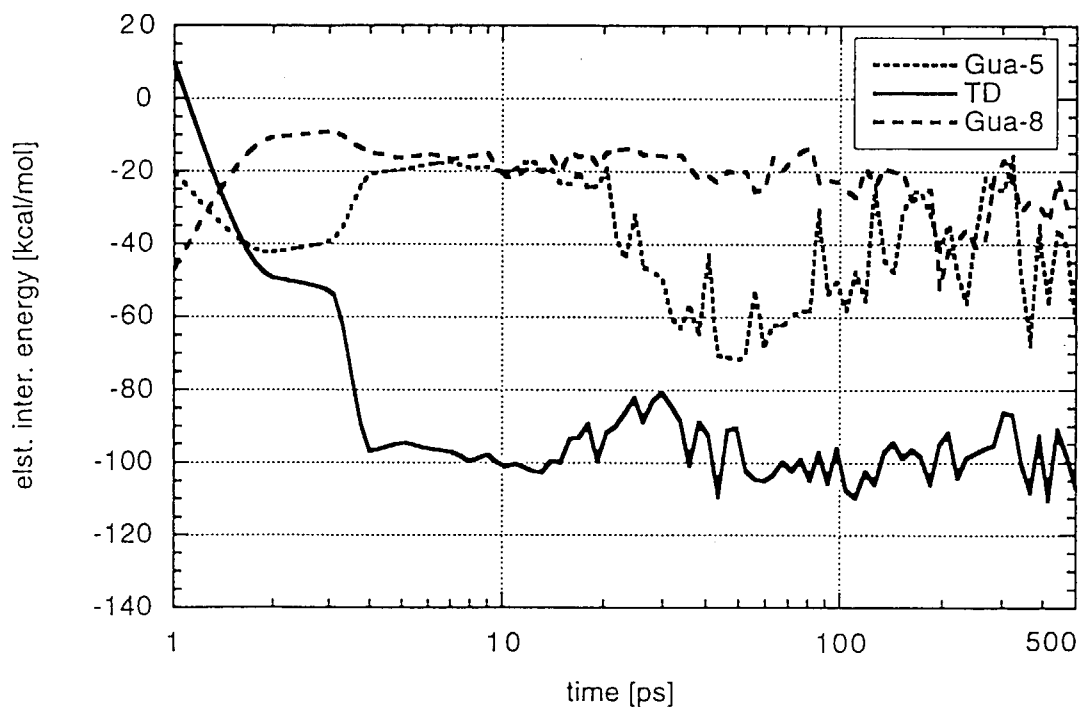


Fig. 10

Electrostatic interaction energy between part of enzyme and respective units of DNA - nucleotides Gua-5 and Gua-8, and TD. The x-axis is logarithmic to clearly display the development of interaction energy in the beginning of simulation, when the complex is formed. At the beginning, the interaction energy has positive value of around +10 kcal/mol (attraction), later when the part of enzyme approaches DNA, it becomes negative (repulsion). The TD is the closest part of DNA to amino acids of enzyme, therefore the repulsion is strongest for TD. After formation of complex (~100 ps), the interaction energy keeps stable.

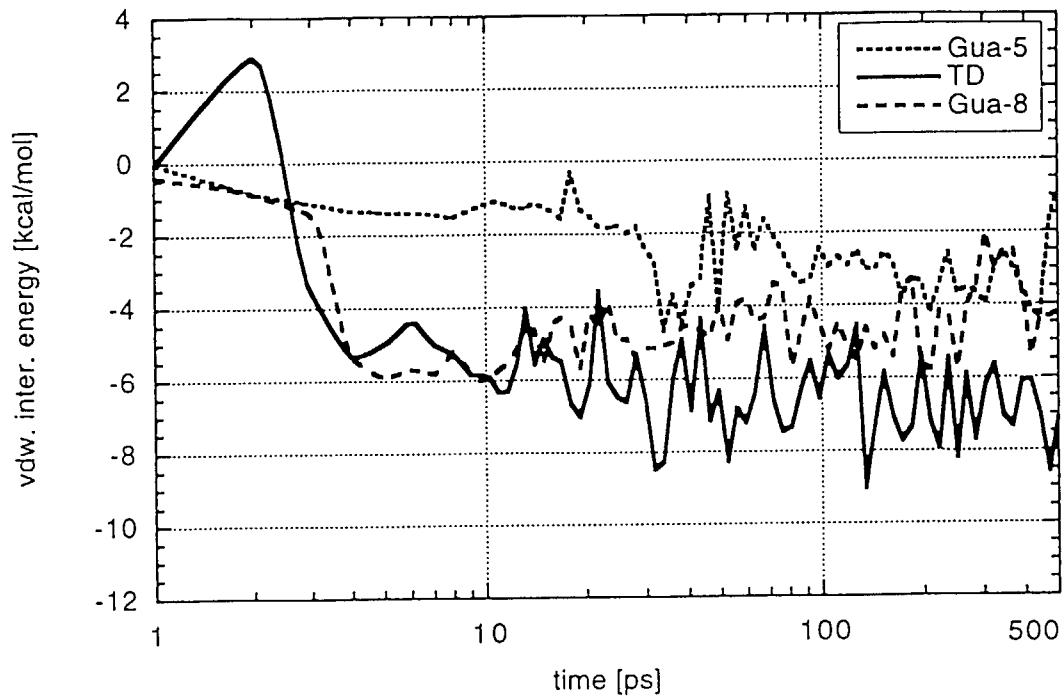


Fig. 11

Van der Waals interaction energy between part of enzyme and respective units of DNA - nucleotides Gua-5 and Gua-8, and TD. Interaction energy is positive between TD and part of enzyme in the beginning of simulation, then becomes negative. This repulsion is stronger for TD than for nucleotides Gua-5 and Gua-8.

国際単位系 (SI) と換算表

表1 SI基本単位および補助単位

量	名称	記号
長さ	メートル	m
質量	キログラム	kg
時間	秒	s
電流	アンペア	A
熱力学温度	ケルビン	K
物質質量	モル	mol
光度	カンデラ	cd
平面角	ラジアン	rad
立体角	ステラジアン	sr

表3 固有の名称をもつSI組立単位

量	名称	記号	他のSI単位による表現
周波数	ヘルツ	Hz	s ⁻¹
力	ニュートン	N	m·kg/s ²
圧力, 応力	パスカル	Pa	N/m ²
エネルギー, 仕事, 熱量	ジュール	J	N·m
工率, 放射率	ワット	W	J/s
電気量, 電荷	クーロン	C	A·s
電位, 電圧, 起電力	ボルト	V	W/A
静電容量	ファラド	F	C/V
電気抵抗	オーム	Ω	V/A
コンダクタンス	ジーメンズ	S	A/V
磁束	ウェーバ	Wb	V·s
磁束密度	テスラ	T	Wb/m ²
インダクタンス	ヘンリー	H	Wb/A
セルシウス温度	セルシウス度	°C	
光束	ルーメン	lm	cd·sr
照射度	ルクス	lx	lm/m ²
放射能	ベクレル	Bq	s ⁻¹
吸収線量	グレイ	Gy	J/kg
線量当量	シーベルト	Sv	J/kg

表2 SIと併用される単位

名称	記号
分, 時, 日	min, h, d
度, 分, 秒	°, ', "
リットル	l, L
トン	t
電子ボルト	eV
原子質量単位	u

1 eV = 1.60218 × 10⁻¹⁹ J
1 u = 1.66054 × 10⁻²⁷ kg

表4 SIと共に暫定的に維持される単位

名称	記号
オングストローム	Å
バ	b
バ	bar
ガリ	Gal
キュリー	Ci
レントゲン	R
ラド	rad
レム	rem

1 Å = 0.1 nm = 10⁻¹⁰ m
1 b = 100 fm = 10⁻²⁸ m²
1 bar = 0.1 MPa = 10⁵ Pa
1 Gal = 1 cm/s² = 10⁻² m/s²
1 Ci = 3.7 × 10¹⁰ Bq
1 R = 2.58 × 10⁻⁴ C/kg
1 rad = 1 cGy = 10⁻² Gy
1 rem = 1 cSv = 10⁻² Sv

表5 SI接頭語

倍数	接頭語	記号
10 ¹⁸	エクサ	E
10 ¹⁵	ペタ	P
10 ¹²	テラ	T
10 ⁹	ギガ	G
10 ⁶	メガ	M
10 ³	キロ	k
10 ²	ヘクト	h
10 ¹	デカ	da
10 ⁻¹	デシ	d
10 ⁻²	センチ	c
10 ⁻³	ミリ	m
10 ⁻⁶	マイクロ	μ
10 ⁻⁹	ナノ	n
10 ⁻¹²	ピコ	p
10 ⁻¹⁵	フェムト	f
10 ⁻¹⁸	アト	a

(注)

- 表1-5は「国際単位系」第5版, 国際度量衡局 1985年刊行による。ただし, 1 eV および 1 uの値は CODATA の1986年推奨値によった。
- 表4には海里, ノット, アール, ヘクタールも含まれているが日常の単位なのでここでは省略した。
- barは, JISでは流体の圧力を表わす場合に限り表2のカテゴリに分類されている。
- EC閣僚理事会指令では bar, barn および「血圧の単位」 mmHg を表2のカテゴリに入れている。

換 算 表

力	N (=10 ⁵ dyn)	kgf	lbf
	1	0.101972	0.224809
	9.80665	1	2.20462
	4.44822	0.453592	1

粘 度 1 Pa·s (= N·s/m²) = 10 P (ポアズ) (g/(cm·s))
動粘度 1 m²/s = 10⁴ St (ストークス) (cm²/s)

圧	MPa (=10 bar)	kgf/cm ²	atm	mmHg (Torr)	lbf/in ² (psi)
	1	10.1972	9.86923	7.50062 × 10 ³	145.038
力	0.0980665	1	0.967841	735.559	14.2233
	0.101325	1.03323	1	760	14.6959
	1.33322 × 10 ⁻⁴	1.35951 × 10 ⁻³	1.31579 × 10 ⁻³	1	1.93368 × 10 ⁻²
	6.89476 × 10 ⁻³	7.03070 × 10 ⁻²	6.80460 × 10 ⁻²	51.7149	1

エネルギー・仕事・熱量	J (=10 ⁷ erg)	kgf·m	kW·h	cal (計量法)	Btu	ft·lbf	eV	1 cal = 4.18605 J (計量法)
	1	0.101972	2.77778 × 10 ⁻⁷	0.238889	9.47813 × 10 ⁻⁴	0.737562	6.24150 × 10 ¹⁸	= 4.184 J (熱化学)
	9.80665	1	2.72407 × 10 ⁻⁶	2.34270	9.29487 × 10 ⁻³	7.23301	6.12082 × 10 ¹⁹	= 4.1855 J (15 °C)
	3.6 × 10 ⁶	3.67098 × 10 ⁵	1	8.59999 × 10 ⁵	3412.13	2.65522 × 10 ⁶	2.24694 × 10 ²⁵	= 4.1868 J (国際蒸気表)
	4.18605	0.426858	1.16279 × 10 ⁻⁶	1	3.96759 × 10 ⁻³	3.08747	2.61272 × 10 ¹⁹	仕事率 1 PS (仏馬力)
	1055.06	107.586	2.93072 × 10 ⁻⁴	252.042	1	778.172	6.58515 × 10 ²¹	= 75 kgf·m/s
	1.35582	0.138255	3.76616 × 10 ⁻⁷	0.323890	1.28506 × 10 ⁻³	1	8.46233 × 10 ¹⁸	= 735.499 W
	1.60218 × 10 ⁻¹⁹	1.63377 × 10 ⁻²⁰	4.45050 × 10 ⁻²⁶	3.82743 × 10 ⁻²⁰	1.51857 × 10 ⁻²²	1.18171 × 10 ⁻¹⁹	1	

放射能	Bq	Ci
	1	2.70270 × 10 ⁻¹¹
	3.7 × 10 ¹⁰	1

吸収線量	Gy	rad
	1	100
	0.01	1

照射線量	C/kg	R
	1	3876
	2.58 × 10 ⁻⁴	1

線量当量	Sv	rem
	1	100
	0.01	1

MOLECULAR DYNAMICS OF FORMATION OF TD LESIONED DNA COMPLEXED WITH REPAIR ENZYME-ONSET OF THE ENZYMATIC REPAIR PROCESS