Sequence Dependent Localized Distribution of Various Water Dynamics in the Grooves of DNA

A thesis submitted towards partial fulfillment of

BS-MS dual degree programme

(August 2012 - April 2013)

By

Shreyas Supekar

Department of Chemistry
Indian Institute of Science Education and Research
Pune – 411021
INDIA

CERTIFICATE

This is to certify that this dissertation entitled **"Sequence Dependent Localized Distribution of Various Water Dynamics in the Grooves of DNA"** towards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune submitted by Shreyas Supekar carried out by the candidate at the Indian Institute of Science Education of Research (IISER), Pune, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or institution.

08th April, 2013

Dr. Arnab Mukherjee

(Research Supervisor)

Assistant Professor, IISER Pune

Pune-411008, India

Declaration

I hereby declare that the thesis entitled **"Sequence Dependent Localized Distribution of Various Water Dynamics in the Grooves of DNA"** submitted for the partial fulfillment of the BS-MS dual degree programme at Indian Institute of Science Education of Research (IISER), Pune has not been submitted by me to any other University or Institution. This work was carried out at the Indian Institute of Science Education of Research (IISER), Pune, India under supervision of Dr. Arnab Mukherjee.

April 8th, 2013 Shreyas Supekar

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BS-MS, IISER Pune

Pune - 411008

Acknowledgements

First, I would like to thank my mentor, Dr. Arnab Mukherjee for his advice, guidance and encouragement at all times. I would always be indebted to him for keeping faith in me, keeping me motivated and helping me develop a scientific outlook. He has been a constant source of inspiration for me.

I would also like to thank all the lab members (Wilbee, Mandar, Sathish and Reman) from Arnab Mukherjee lab. I learnt a lot from them all. Especially Wilbee D. S., in gratitude for countless times she has helped me unconditionally. Thank you for being ever supportive.

It would not have had been possible without my friends at IISER. You guys provided me constant shipment for fun and joy with a pinch of salt at times. This experience would have had been incomplete without you.

Zino Davidoff for helping me stay awake for a many a night.

I would never have had walked this road if it was not for my family. Thank you for endless trust and support.

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Abstract

Water dynamics in the solvation shell around biomolecules plays a vital role in the stability, function and recognition processes. Although extensively studied, ambiguity still exists over the timescales of relaxation, extent of water slowdown, and sequence dependence of water dynamics obtained from different experiments. We have performed molecular dynamics simulations of 20 DNA systems containing all possible dinucleotide steps in adequate statistics. We have calculated residence times, orientational relaxation time and diffusion coefficients around each of 360 different dinucleotide steps which provided a distribution of timescales of the above properties around the major and minor grooves of DNA. While for most of the water molecules, dynamics follow a Gaussian distribution, a slow tail exists for some. Decomposing the timescales for each dinucleotide steps shows a varying scatter over significant time range for each indicating presence of an underlying topological effect along with the chemical nature of the DNA. No notable difference in the re-orientation mechanism of water was found around DNA compared to bulk. The fact that even with the same basic unit of the DNA, a dinucleotide step, one gets a range of timescales for various water dynamics indicates the underlying reason for the ambiguity in timescales obtained from different experiments. This study thus points to the inherent heterogeneity of bimolecules dictated by both structure and chemistry and calls for a fresh perspective in terms of recognition processes that involves the inherent role of waters.

1. Introduction

Importance of Water. Water has been referred to as the "matrix of life"[1-2], "elixir of life"[3] and rightly so, given its ubiquity and importance in all biomolecular systems. The function and stability of biomolecules like nucleic acids and proteins is entirely lost in absence of water. Yet, the role of water in biomolecular dynamics had been underplayed in previous years. It is becoming more and clearer that water plays an active role in biomolecular recognition and dynamics rather than previously held notion of it being a passive solvent. Thus, an understanding of dynamics of water molecules in solvation shells of biomolecules, also known as biological water[4] is essential to the understanding of biomolecule stability, behavior, function and recognition.

DNA Structure. One of the most important biomolecular system is the Deoxyribose Nucleic Acid (DNA), landmark discovery of which in 1953 by Watson and Crick[5] remains to be one of the most historic moments in the history of science and led to huge advances in fields like molecular biology and biochemistry. DNA is a polymer of nucleotides (Fig. 1a) Adenine(A), Thymine(T), Cytosine(C) and Guanine(G). Each nucleotide consists of a 5-carbon sugar (deoxyribose), a nitrogenous base attached to the sugar and a phosphate group. A, T, G and C differ only in the bases they constitute of. A and G are purines while T and C are pyrimidines. The nucleotides are chained together by phosphodiester links between the two consecutive sugars. DNA occurs normally as a double helix. The two chains run in opposite directions (Fig. 1a). The bases are located in the inner part of the helix. Three-dimensional structure of DNA looks like helical staircase (Fig 1b). The phosphate groups are the most exposed parts of DNA being the farthest from central axis. DNA contains two grooves – the one towards the backbone is called minor groove, while the one on the opposite side is called major groove. Even at the base pair level, one can identify major groove and minor groove side of the base pair as shown in Fig. 2.

DNA-Hydration. DNA hydration is very important for its conformation and functionality.[5-7] The hydration of DNA is more pronounced than proteins due to ionic interactions of

the electronegative atoms of DNA with aqueous environment. The polymorphic nature of DNA is controlled by the amount of water. Under normal physiological condition, DNA takes B-form[8-9], which has a narrow and deep minor groove, and a shallow and wide major groove (opposite in case of A-DNA).

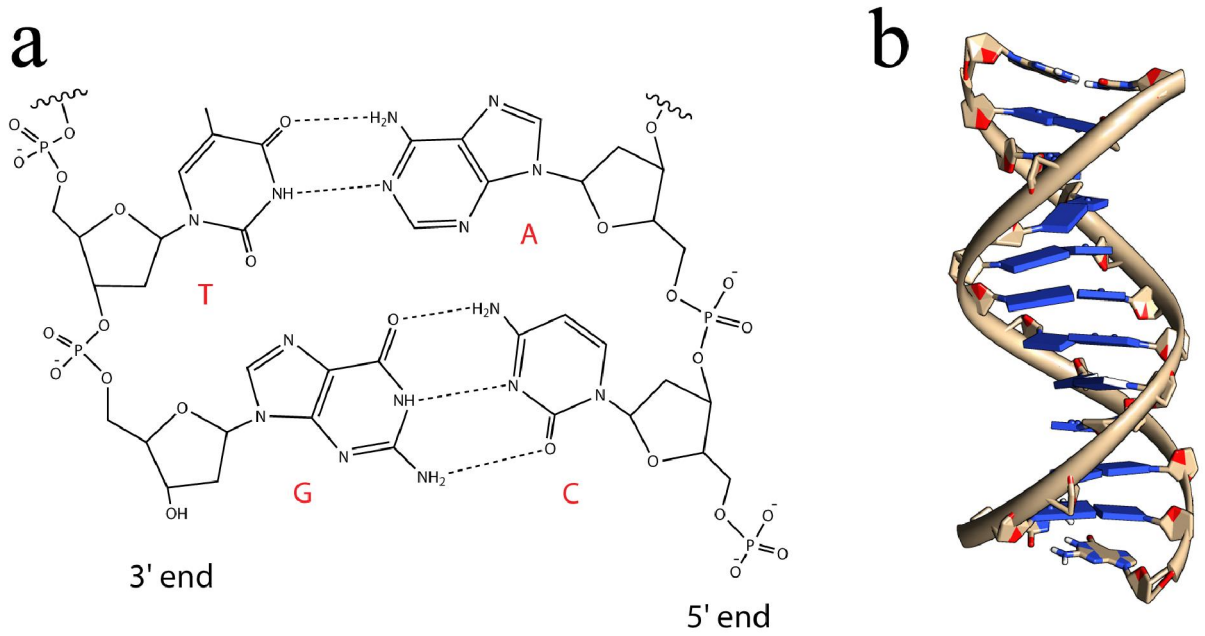

Figure 1: Structure of DNA. (a) chemical structure (b) 3-dimensional structure.

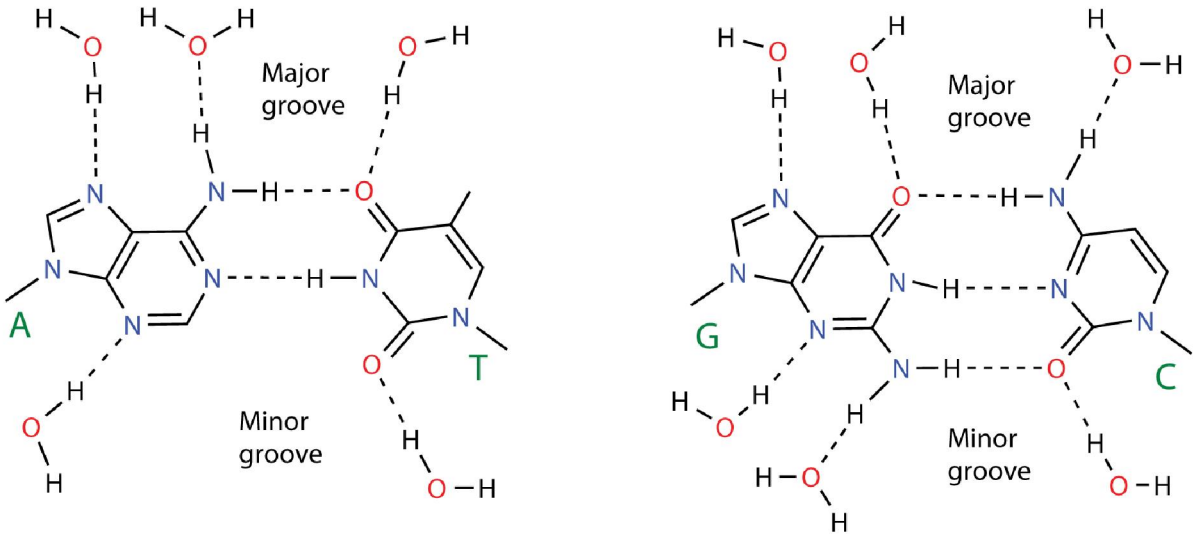

Figure 2: AT and GC basepairs of DNA. Hydrogen bonding patterns and locations of major and minor grooves for the basepairs(left and right respectively).

Water molecules are more strongly held around phosphates through hydrogen bonding but they don't stay too long here[10], attributing to highly diffuse nature of charge distribution on phosphate groups[10]. Water molecules are more ordered and stay for longer around bases due to presence of directional hydrogen bond acceptors (Fig. 2) and constrained volume imposed by the grooves around the bases. Deep and narrow architecture of the B-DNA minor groove results in formation of a "spine of hydration" B-DNA as shown in Fig. 3a.This was first shown by Dickerson and coworkers[11-12] in A/T rich sequence. Subsequent research on spine of hydration points to the fact that spine of hydration is the primary reason for the narrowness of minor groove in A/T rich sequences.[13] Theoretical studies also suggest the possibility of the existence of spine of hydration in G/C rich sequences too.[14-15] Major groove is wider and shallower. Therefore, the spine does not form (Fig. 3b). These water molecules present in grooves play a key role in DNA stabilization and recognition[16]. Some of these deep seated waters serve as a navigator and provide contact points for incoming protein or drug[17].

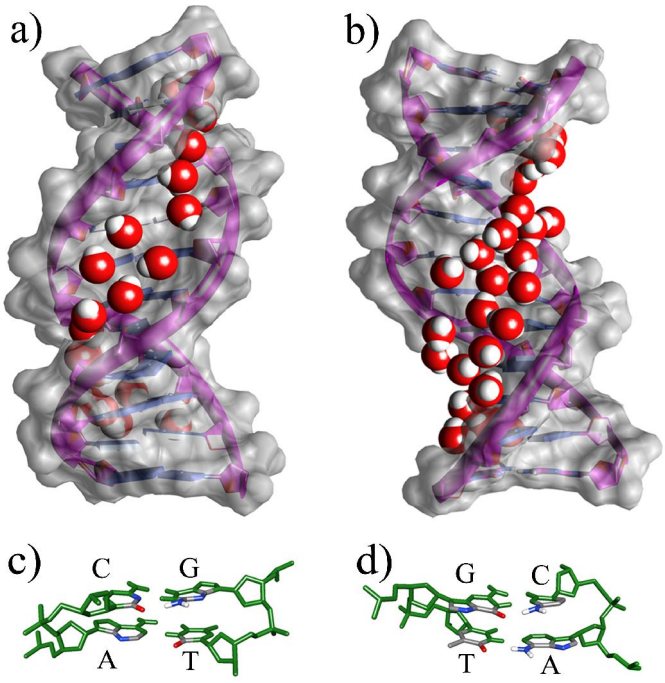

Figure 3: Water moleules hydrogen bonded to groove atoms. (a) Minor groove surface waters (b) Major groove surface waters (c) Atoms on the surface of minor groove (d) Atoms on the surface of major groove.

Properties of Water. Many of the interesting properties of water stem from the constantly fluctuating hydrogen bond network of liquid water. In liquid state, water exists as constantly fluctuating network of intermolecular H-bonds. One water molecule can participate in up to 4 hydrogen bonds. It can accept up to two and donate up to two hydrogen bonds. However, waters in the solvation shells of biomolecules (biological water) differ from that of bulk water in both their statics and dynamics. As Philip Ball elegantly stated in his review on water: The shape and size of the biological water depends on how biomolecules manipulates its solvation shell. Also, there is a feedback from the solvation shell to the biomolecule which regulates the biomolecule's biological function in turn.[18] Hence one can say that, biological function of a biomolecule depends on the subtle interplay between solvation shell water molecules and the biomolecule. For example, water in the minor and major grooves of the DNA play important roles in protein-DNA and drug-DNA recognition (binding) processes. Therefore, understanding the dynamics of water in the grooves will throw light into the dynamics and functioning of these recognition processes.

Various Water Dynamics. For water molecules in the hydration shell, relaxation processes are: first, the liberation and vibration of water molecules which occurs due to disruption of hydrogen bond network among water molecules and corresponds to the initial ultrafast decay.[17] Second, the bulk water-like relaxation which corresponds to intermediate timescales of relaxation. This occurs by the water molecules which although are present in the solvation shell yet do not form H-bond to the biomolecule. Third, the hindered relaxation, which occurs due to reduction in degrees of freedom of a water molecule either via strong hydrogen bonding or by reduction of accessible volume. Fourth, the ultraslow timescale which is said to originate from the relaxation of the biomolecule itself[19].

Probing Water Dynamics Using Experiments and Theory. In the last two decades there have been innumerable experimental and theoretical studies on dynamics of water molecules in the solvation shell of biomolecules. The dynamics of hydration shell

water molecules can be probed by examining various relaxation times. Relaxation can be defined as the process during which a perturbed system returns back to its equilibrium state. A relaxation process can be characterized by its relaxation time. The relaxation time gives us an insight into how fast or slow the reorganization of the systems occurs so as to attain equilibrium. For example, in a typical dielectric relaxation for a biomolecular system, a probe is excited which leads to change in electric field. This is followed by spatial reorganization of solvent molecules and solute locally. Such a relaxation is measured and it gives us information about how fast or slow the system reorganizes when subjected to small perturbations. However, still after two decades of extensive research, the relaxational timescales obtained from various experimental and theoretical studies and the exact role of biological water in biomolecular hydration remains debated[20]. In case of experiments for biological systems, different experiments study probe dynamics on different scales of length and time.[21] For example, NMR is sensitive to very short length scales (few angstroms) while dielectric relaxation averages all length scales. Also, even for the same kind of experiments, different values have been reported. For example, using NMR, Wuthrich *et al.* reported water relaxation timescales in the solvation shell of proteins in the range 300-500 ps[22] whereas another research group report using NMR reported relaxation timescales in the range 10 – 200 ps[23]. This behavior is similar to water molecules found in the solvation shell of proteins[24]. Also, they reported residence times longer than 1ns for waters in the minor groove. These waters correspond to inner waters residing on the surface of minor groove.[25] NMR studies by Denisov and coworkers suggest that the long relaxation timescale found for water molecules in the solvation shell of DNA arise primarily due to spine of hydration in minor groove.[26] Dielectric relaxation experiments using fluorescent probe, on the other hand, report a time scale of the order of 10 ns.[27] *It is clear that there is a disparity of orders of magnitudes in the reported timescales.* This renders theoretical evaluation as the best approach as theoretical studies overcome experimental issues like position of the fluorescent probe which influences the kind of water molecules they would be probing as relaxation measurements are a result of reorganization local environment of the probe. In theoretical studies, relaxation measurements are done by calculating various autocorrelation functions. For example,

normalized velocity-velocity autocorrelation function on integration gives us the diffusion coefficient for the system.

Recently, the origin of slow dynamics of water molecules in the vicinity of biomolecules has seen resurgence. As mentioned previously, there have been discrepancies in the timescales reported using NMR and dielectric relaxation experiments due to their systemic shortcomings. These days TRSS (time resolved stokes shift) spectroscopy is seen as the best technique to probe the dynamics of hydration. In case of DNA, TRSS overcomes the shortcomings of other spectroscopic techniques as in TRSS, the probe sits between the base pairs and hence it efficiently captures the fluctuations near all local fragments of DNA namely, the phosphate oxygens, the water molecules, the base pairs and ions present in the local environment of the grooves. Berg and coworkers used coumarin-102 dye as a probe attached to deoxyribose ring of DNA. They recorded timescales from 40 ps – 40 ns for relaxation around DNA. They also reported no sequence dependence on relaxation timescales.[28]

Sen and coworkers tried to replicate results obtained from TRSS using molecular dynamics simulations. They were unsure of the origin of different timescales in TRSS there exist cross correlations from various sources. These timescales could be due to perturbed water, DNA motion or counter ions or a coupling of these three in a complex way. Hence they used simulations to circumvent the issues originating due to experimental perturbation of the system. According to the authors, from 1-100 ps timescale water has the major contribution to relaxation. From 100 ps onwards the ions start contributing but there is no DNA contribution whatsoever. From these results, one can infer that water is the most important player in relaxation at all times.[20] Contrastingly, Furse and coworkers performed MD simulations on DNA bound to Hoechst system and gave exactly opposite conclusions. They reported that DNA is the major contributor to long time relaxation rather than water whose contribution to long timescale is negligible.[19] One can clearly see that the literature is rife with contradictions of this kind and there is still no consensus on various issues in biomolecular hydration dynamics.

Drug-DNA recognition. Water molecules in the solvation shell of biomolecules play a crucial role in its recognition and it is extensively shown by many research groups over

the past few years[29-31]. A study by Zewail et al., with femtosecond time resolution[29] probed the dynamics of wetting/dewetting with DNA bound to a drug Hoechst 33258. The drug was bound to two different DNA sequences, Dickerson dodecamer which has an AT rich core and Calf thymus DNA which has a GC rich core. The authors observed bimodality in relaxation time scales of water molecules in the solvation shell of DNA-drug complexes. The authors stated that although recognition of DNA occurs via complementary charge distributions, complementary topologies and via hydrogen bonded contacts between a drug or a protein, the mechanism of DNA recognition can't be understood without considering the contribution of water molecules at the DNA surface. The initially ordered water gets disrupted in the process of drug binding to the DNA and this disruption of ordering is essential in recognition of DNA by drugs and other DNA binding molecules. Also, sequence specific recognition of DNA by small molecules has been reported.[30] Ha *et al.* studied the thermodynamics of water release during specific recognition of DNA by DNA binding proteins and they found that such an expulsion of solvation shell water molecules is essential for sequence specific recognition.[31] There have been great number of studies on similar lines which make it clear by the sheer amount of evidence amassed that sequence specific recognition of DNA is heavily influenced by water molecules in its solvation shell.

Recently, entropy calculations by Bagchi *et al.* for water molecules in the major and minor grooves of DNA using translational and rotational diffusion coefficients obtained from molecular dynamics simulations of DNA suggest reduction in entropy of water molecules in solvation shell as compared to bulk water. Furthermore, the entropy in the minor grooves was found to be lower than that in major groove.[32]

Water Dynamics around Other Systems. There have been studies on water dynamics near proteins and a distribution of relaxation times has been reported. *These studies show an inherent heterogeneity in distribution of dynamical properties* as well as energetics of water across various sites of the biomolecule.[33-34] Such a behavior may be the consequence of both, the chemistry and the topology of the site of hydration. In a DNA system, the sites may not vary much on a topographic scale but chemically, it has been known of having quite a varied behavior, based on the nucleobases it constitutes

of, and their sequential order of arrangement. Fayer and coworkers have performed experimental studies to observe slowdown of water molecules in the solvation shell using Infrared pump probe experiments and two-dimensional IR spectroscopy on ionic reverse micelles and have compared the results with planar lamellar interfaces. Both the structures were formed using same monomer units. They have suggested that presence of an interface is most essential to slowdown rather than chemistry or topology of it.[35] Many studies on the other hand suggest heavy dependence of sequence on water dynamics in the solvation layer.[36] The greater volume of studies on hydration dynamics is done for protein systems and there remain still untended questions in DNA hydration dynamics. *As rightly pointed out by Szyc et al., the study of role of water plays in the properties of nucleic acids, though extremely important but is highly neglected in comparison to proteins which are extensively studied.*[37]

Why this study? The dynamics of biological water are bound to change with a change in the sequence of DNA and hence, an investigation at a base-pair level could shed more light on sequence effects of DNA on water dynamics in its solvation shell. This sequence dependence can be probed by looking at water dynamics around 10 unique dinucleotide steps. It is well known that AT-rich sequences are B-phillic and GC-rich sequences are A-phillic. A better knowledge of sequence dependence on solvation water dynamics might also help predict A or B-phillic nature of any arbitrary DNA sequence.

Goal of the Present Study. Here, we attempt to investigate heterogeneity in water dynamics around dinucleotide steps in order to understand sequence dependence on water dynamics in solvation shell of DNA. We have done molecular dynamics simulations of 20 DNA systems immersed in water and analyzed residence times, orientational correlation times and velocity autocorrelation functions for water molecules in the first solvation shell of DNA. We have mapped the distribution around major and minor groove segments of all the dinucleotide steps encompassing the 20 sequences. We selected the groove portions of dinucleotide step as a basic unit for analysis. A dinucleotide step is pair of consecutive base pairs when moving along the DNA (Figs.

3c, 3d). We selected these as a basis for our analysis as dinucleotide steps are the most basic and simplest possible units of a DNA molecule, which help shedding light on the sequence dependence on water dynamics of a lack thereof.

2. Methods

2.1 Sequence Generation

Four hundred random DNA dodecamer sequences of the type, d(CGXXXXXXXXCG) were generated where X represents a random base, A, T, C or G. Frequency of the 10 uniquely possible dinucleotide steps was calculated for all the sequences. 20 sequences from the 400 generated sequences were selected such that the frequencies of dimers follow a more or less uniform distribution. Furthermore, to improve the uniformity of the distribution, the two terminal dinucleotide steps name the two CGs were flipped to GC in all possible combinations and frequency calculation was done again for all such sequences. Again, one among the possible four combinations for each of the 20 were selected as the final sequences. The final selection of 20 DNA structures follows uniform distribution for the frequency of dinucleotide steps (Fig. 4) These DNA sequences were processed using NAB program[38] to get structure files. AMBER99/parmbsc0 force field[39] was used for generating the topology and coordinate files. The coordinates and topologies obtained were converted to GROMACS[40] format using amb2gmx.pl program [41].

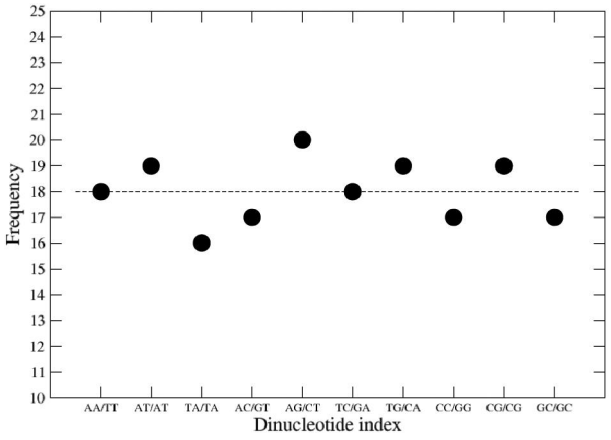

Figure 4: Frequency distribution dinucleotide steps in all 20 sequences.

2.2 Simulation Details

The 20 DNA systems obtained were solvated using TIP3P[42] water molecules. The box dimensions were chosen such that there was a 1nm thick solvation shell around each DNA system. The system was neutralized by replacing 22 water molecules by 22 Sodium ions. These DNA systems were energy minimized using steepest descent method[43] followed by heating up to 300K using a Berendsen thermostat[44] with a coupling constant of 0.2ps and a harmonic restraint of 2.5kcal/mol/Å^2 on heavy atoms of DNA. This was followed by a gradual sequential reduction of restraint to 50 cal/mol/Å^{2}in 7 simulations, 50 ps each at constant temperature 300K and constant pressure 1bar using Berendsen thermostat and barostat[44] respectively. The final equilibration without any position restraints was carried out for 1ns with a time step of 2fs at constant temperature 300K and constant pressure 1bar using Nose-Hoover thermostat[45-46] and Parrinello-Rahman barostat[47] respectively with a coupling constant of 0.2ps. Electrostatic interactions were treated using PME electrostatics[48] with 10 Å as cutoff. Van der Waals cutoff was set as 10 Å. A final molecular dynamics run of 10ns with a time step of 2fs was carried out for all the 20 DNA systems with similar treatment of temperature, pressure, electrostatics and vdW as in final equilibration. All the molecular dynamics treatment was done using GROMACS package.[40]

2.3 Theoretical Section. The theoretical background of the various dynamical quantities is introduced here.

2.3.1 Residence time. The residence time is the average amount of time a water molecule stays in the solvation shell of a biomolecule. Originally, in strictly theoretical terms, residence time can be calculated by measuring on an average, the time a water molecule spends continuously in a certain region. However, this underestimates the real residence time as a water molecule can escape and return to the particular region due to re-crossing. Subsequently, to overcome the drawback, a time lag was allowed to come back to the region once a water molecule escapes. However, the time lag was

arbitrarily assumed to be 2 ps for various systems. Therefore, Laage and Hynes prescribed a stable states picture (SSP)[49]. Under the SSP prescription (Fig. 5), we define a stable reactant state as a water molecule being within the first solvation shell of the reference site and stable product state as it being out of the first solvation shell. The "stable state" refers to a state from which a trajectory can't escape for a long time. For such a two-state system we define regions beyond which system lies in a stable state. Such a definition of stable states takes care of transient escapes or re-crossing events.

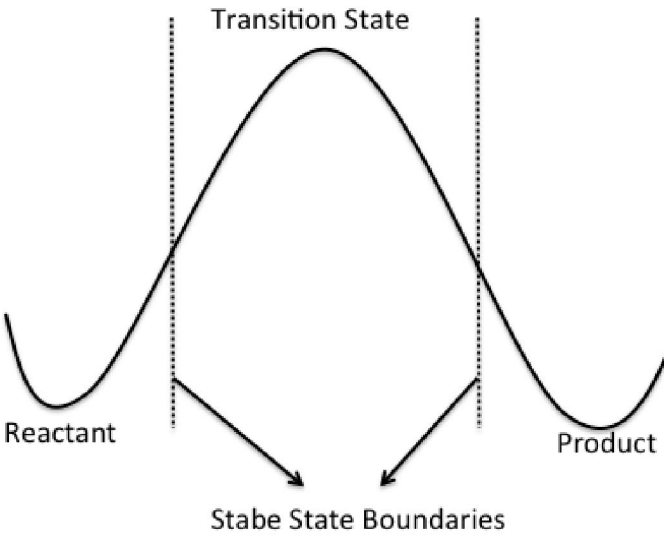

Figure 5: Schematic diagram showing the stable state picture (SSP) criteria. Dashed line denotes the boundary beyond which the system goes to stable state (reactant and product) and cannot re-cross.

Mathematically, the residence time via SSP prescription may be given as the decay time of the compliment of the probability of a trajectory to be in stable reactant state at time t=0 and to be in stable product state at time t=t. The SSP procedure can be summarized as,

$$C(t) = 1 - \langle p_R(0).p_P(t)\rangle = e^{-t/\tau_{SSP}^{res}}, \qquad (1)$$

where, p_R and p_P are the probabilities of being in stable reactant and product states respectively.[49]

This definition was used to write an analysis program in C programming language using GROMACS[40] libraries. The program primarily operates on the trajectory files obtained from the simulations to get the mean residence time in the first solvation shell of the residue specified.

The credibility of the program was verified by doing residence time calculations for residence times in major and minor groove (Fig. 6). We obtained 15.35 ps as the intermediate timescale and 119.12 ps as the longer timescale for minor groove. For major groove, we obtained 8.19 ps as the intermediate timescale and 87.5 ps as the longer timescale. The values obtained were found to be in line with the experimental and theoretical residence times reported in the literature.

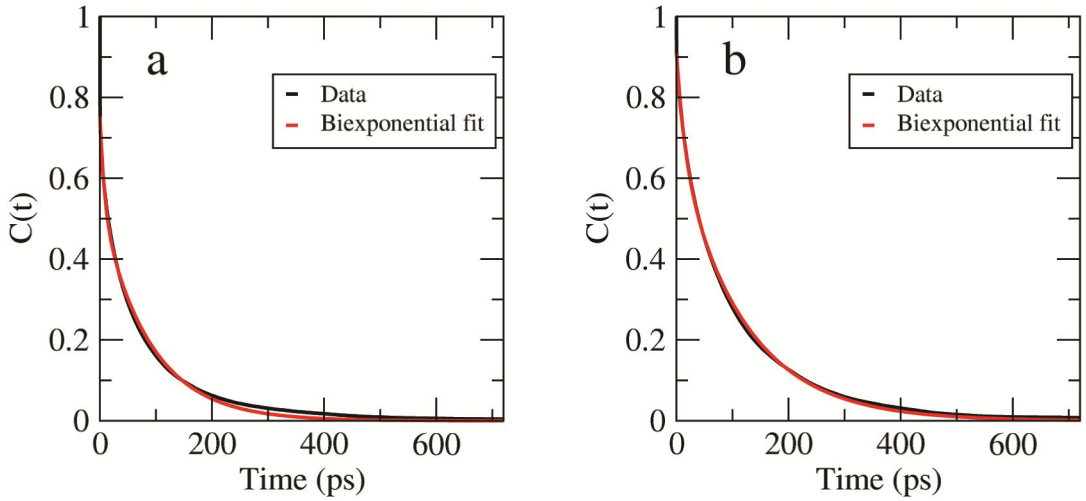

Figure 6: Residence time plots (a) Major groove (b) Minor groove.

The residence times were calculated for the minor and major groove component of each dinucleotide step (9 such steps leaving the terminal base pairs) for each DNA system (20 systems) while excluding the terminal dinucleotide steps as these are open to water at the ends. The averaging was done for 720ps over 5ns segment of the DNA system trajectory. For both major and minor grooves, thus, we generated 360 correlation plots for water residence times around a dinucleotide.

2.3.2 Orientational correlation function (OCF). OCF of water molecules in solvation shell of a residue gives us how fast on an average, the water molecules in the solvation

shell de-correlate or lose the memory of their previous angular orientations in time. The orientational correlation time can be given by the decay time of probability of water molecule retaining the memory of its orientation in space at time 0 to time t. Mathematically, this can be expressed by the following correlation function,

$$C(t) = \langle P_n[\vec{u}(0).\vec{u}(t)]\rangle = e^{-t/\tau}\,, \qquad (2)$$

Where, $\vec{u}$ denotes the orientation of the vector attached to a water molecule along an OH bond. $P_n()$ refers to n_{th} order Legendre polynomial. Here, we average over second order Legendre polynomial as this gives us a decay comparable to the experimentally measurable quantity, anisotropy.

We calculated OCF of water molecules in the first hydration shell. This means that we also used a distance cutoff (0.325 nm) within which the water molecules need to be there in the course of its orientational relaxation (i.e., between 0 and correlation time t).

This kind of treatment poses the problem of transient re-crossings as discussed earlier. This is definitely leads to some ambiguity for such a treatment for OCF. The problem here is that one can't define a stable reactant and product state in this case. The definition of OCF of water molecules in solvation shell inherently depends on the residence times of the corresponding waters. Once a water molecule escapes the first hydration shell, its environment changes and we do not consider its orientational relaxation (as that would be the orientational relaxation in bulk).

As for residence time, an analysis program in C incorporating GROMACS[40] libraries was written to calculate OCF in the first solvation shell of a residue. As before, the program was tested for bulk water and the resulting values for orientational relaxation times matched with the literature (Fig. 7). A timescale of 2.48 ps was obtained which is in agreement with the values reported for bulk water orientation relaxation in existing literature.

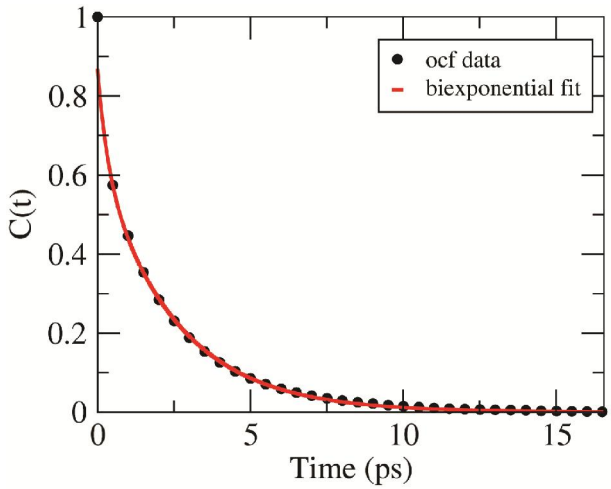

Figure 7: Orientational correlation function for bulk water along with biexponential fit.

Similar to residence times, analysis was done for minor and major groove component of each dinucleotide step for each DNA system while excluding the terminal dinucleotide steps as these are open to water at the ends. Due to memory limitation, the ocf calculations were done for 1ns segments of every 10 ns trajectory, which means we needed to calculate 3600 OCF plots and each OCF is an average of 10 OCFs giving 360 different OCFs similar to the residence time.

2.3.3 Velocity autocorrelation function (VACF). Velocity autocorrelation function measures on an average, how correlated velocity of a particle is at a time t with its velocity at time t=0. The integration of normalized VACF gives diffusion constant, D of molecules of the system under study.

$$C(t) = \langle \vec{v}(0) \cdot \vec{v}(t) \rangle, \quad (3)$$

$$D = 1/3 \int_0^\infty C(t).\,dt \quad (4)$$

Here, we calculate VACF for water molecules in the first solvation shell of the corresponding residue. The position of every water molecule is monitored and if a water molecule stays in the first solvation shell of the residue specified for as long as 4ps continuously, its trajectory snippet is extracted. The averaging of velocity autocorrelation is done over these trajectories. Normalized VACF is then integrated using standard trapezoidal integration procedure to calculate diffusion coefficient [Eq. 4]. Similar to

residence times, 360 VACF plots and corresponding diffusion coefficients were obtained.

As before, the program for calculation of VACF was tested for bulk water and the values obtained were comparable with standard values (Fig. 8).

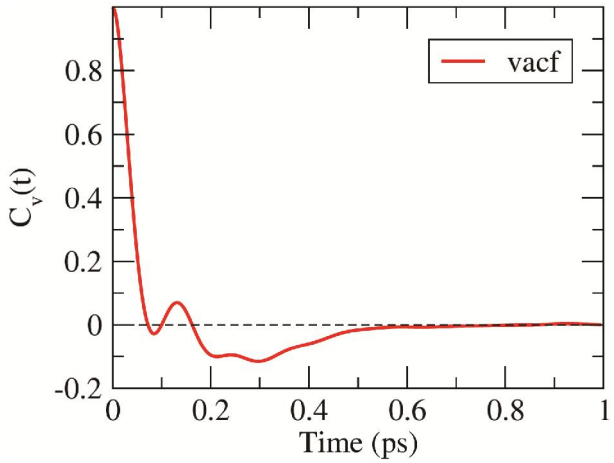

Figure 8: Velocity autocorrelation function for bulk water

2.3.4 Water reorientation mechanism study. Water is now known to orient by jump and diffusion process shown by a seminal paper of Laage and Hynes[50]. We used the same approach described below to study water reorientation mechanism around DNA.

When a water molecules changes its hydrogen bonding partner, a switch occurs. A H-bond here is characterized by standard H-bond definitions[51]: R_{OO}< 0.35nm and θ_{HOO}< 30°, where R_{OO} is the distance between donor and acceptor oxygen atoms, and is the angel between OH and OO bond vectors. During the event of a typical "switch" a donor molecule violates the H-bond definition with a previous acceptor and satisfies the condition with a different acceptor.[50] All such switches were recorded from a 1ns simulation trajectory of Dickerson's dodecamer immersed in DNA. The MD treatment was similar to that presented above for DNA simulations except that the time step of 0.5fs was chosen.

An analysis program was written in C programming language incorporating GROMACS[40] library files which detected all such switches in the first solvation shell of DNA and various coordinates of this "switch reaction" were obtained from the program.

Our main aim was to observe the mechanism of H-bond switch in the first solvation shell of DNA. Every switch event was first recorded throughout the trajectory and then every such switch event was considered individually consisting of the donor along with the former and latter acceptor water molecules. In all the extracted switch trajectory snippets, the moment just before the switch occurs is assigned the time t=0. Various reaction coordinates were extracted ranging from t = -1ps to t = 1ps centered at t=0 for the 3 water molecules (the donor and two acceptor water molecules) in an extracted trajectory snippet. The analysis was done major groove, minor groove and phosphates of the Dickerson's DNA[8]. To test our analysis program, we first reproduced the water reorientation mechanism in bulk water (see later) and subsequently calculated the mechanism of water reorientation around DNA.

3. Results and discussion

3.1 Residence times

Here, we have calculated residence times for major and minor groove components for all 10 dinucleotide steps across all 20 DNA sequences. Fig. 9 shows a typical residence time of water near a dinucleotide step (in this case, AG/CT step) in the minor and major grooves. Here, we have taken 0.35 nm, the radius of first solvation shell of DNA as the cutoff, for selecting water molecules in the first solvation shell. The residence times can be seen as a number depicting average occupation time of water molecules in the first hydration shell of the residue. In principle, it gives us the mobility of waters.

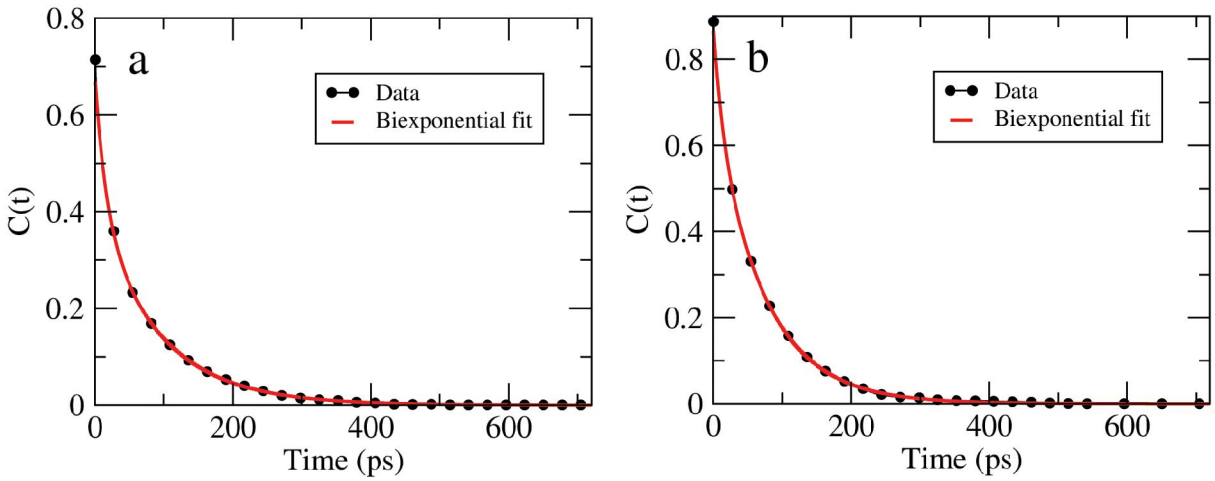

Figure 9: Typical residence time plots near dinucleotide step in the grooves. (a) Major groove (b) Minor groove. The biexponential fit is also shown.

The residence time plot for first solvation shell waters decays slowly due to the presence of electronegative atoms in the grooves, which have a greater strength of hydrogen bond with water as compared to water-water hydrogen bond. The residence times for the water molecules in the minor groove residues tend to be longer than those in major groove residues, as expected. 360 such residence time around major and minor groove correlation function are then fitted with biexponential function of the following form,

$$C(t) = C_1 e^{-t/\tau_1} + C_2 e^{-t/\tau_2}, \qquad (5)$$

where C_1 and C_2 are the weights and τ_1, and τ_2 are the two time scales, the first being fast while second slow timescale. To understand the heterogeneity in the timescale for each given dinucleotide step, we show in Fig. 10 the values of the two timescale for each dinucleotide step around major and minor groove, while in Fig. 11 we show the distribution of the two different timescales in major and minor groove.

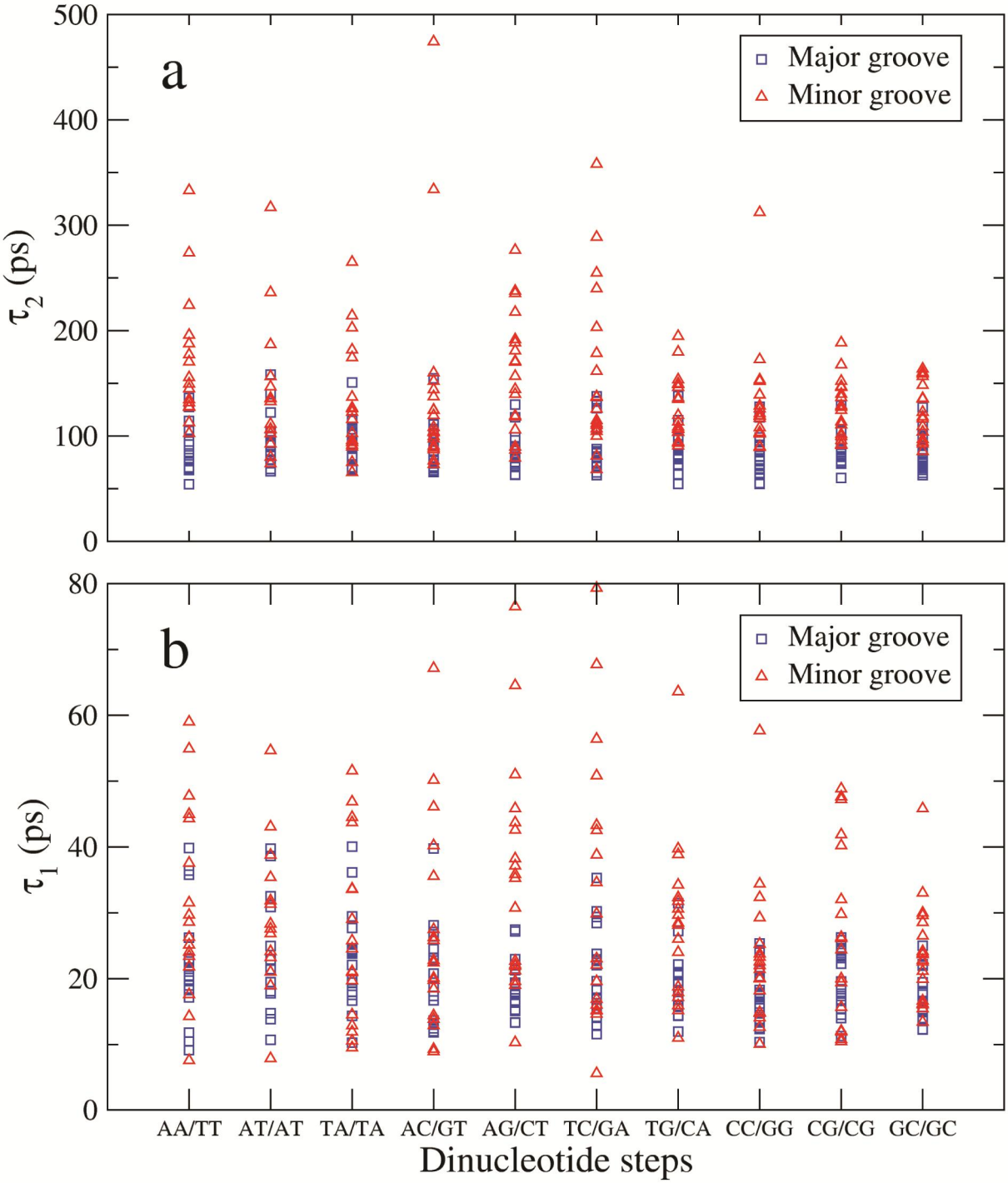

Figure 10: Scatter plot of residence timescales obtained for every dinucleotide step. (a) Slow residence timescales (τ_1) (b) Fast residence timescales (τ_2)

It is evident from a primary inspection of the Fig. 10 that the variation or the spread of the residence times decreases as one moves from A and T dominated steps to C and G

dominated steps for the slow timescale. The segregation with respect to the sequence is less for the fast timescale. Moreover, both the slow and fast timescale is smaller for the major groove compared to the minor groove.

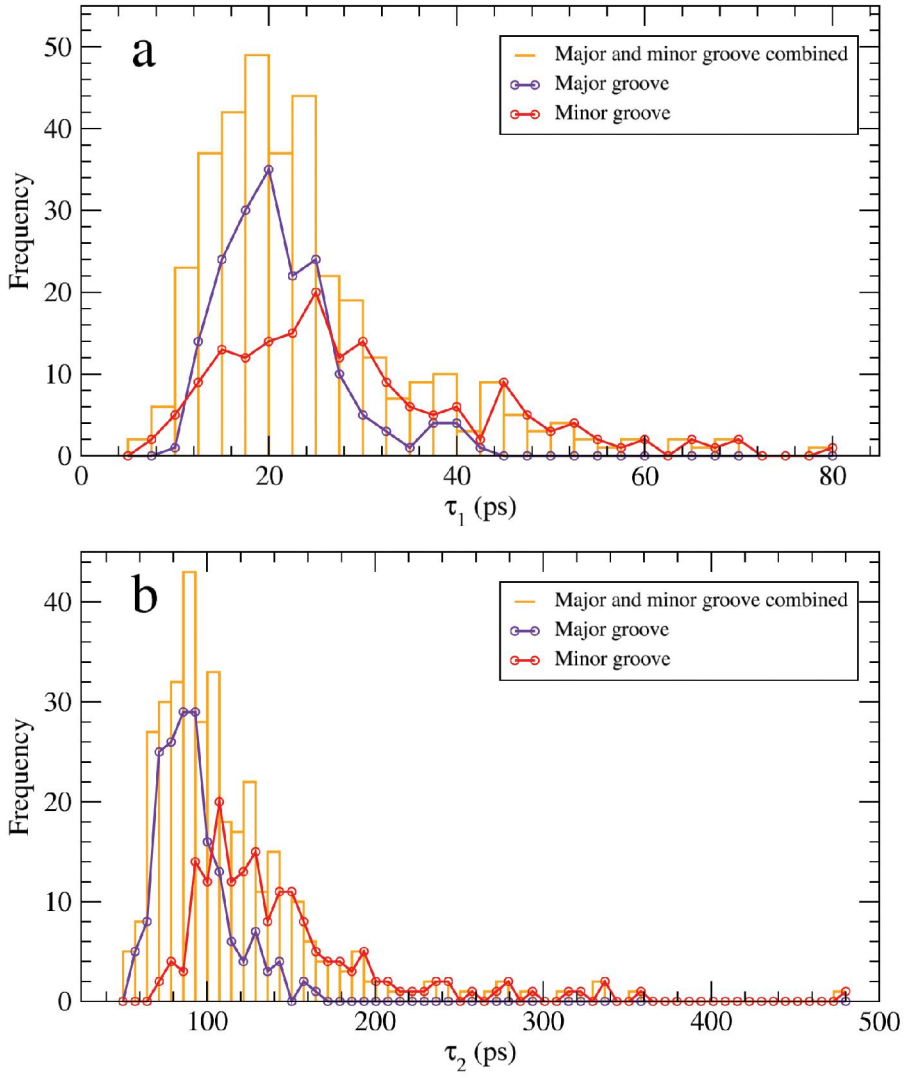

Figure 11: Residence times distribution. (a) Fast timescales (τ_1) (b) Slow timescales (τ_2)

Fig. 11 shows that for both the time scales (slow and fast), the major groove distribution resembles a Gaussian distribution with a tiny tail and the minor groove distribution resembles a Gaussian distribution with a longer tail. This longer tail is primarily responsible for a tail in the distribution of time scales for the whole DNA. The tail points to a heterogeneity of distribution of residence times of water molecules in the first

solvation shell of DNA. It also tells us that the heterogeneity is mainly a result of water molecules in the minor groove part of the hydration shell. Also, the major groove distribution has a higher peak and lesser spread when compared with minor groove whose Gaussian portion of distribution is shorter and more spread. This heterogeneity in the distribution explains the myriad of residence times obtained in experiments ranging from residence time values as high as 500 ps to as small as 20 ps.[22-23] This huge discrepancy of a few orders of magnitudes is a reflection of the heterogeneity resulting from the experiments that tend to probe different regions of DNA. For example, NMR studies give times ranging from 10-500 ps,[22-23] while dielectric relaxation give a time scale of the order of a 10ns.[27] This gigantic value from dielectric relaxation is probably due to the fact that here, the DNA motions also come into picture which results in such huge residence times.

The two timescales obtained here, namely the slow and the fast timescale arises as water molecules in the solvation shell are present in two states, bound and free state. The bound state corresponds to the water molecule H-bonded to one of the electronegative atom of the DNA residue. The states can be imagined as being in equilibrium and hence there is always an exchange of water from one state to another. The water molecules in the free state behave like bulk water molecules whereas the bound state waters which are hydrogen bonded to DNA are deeply seated in the grooves and stay there for a long time. Though, one cannot exactly specify segment of a residence time plot to be exclusively due to bound or free waters except the very tail, which is exclusively due to bound waters, one can say by inspection that the initial part up to a few tens of picoseconds is predominantly due to free state waters while beyond that as the residence time plot decays the contribution is majorly by bound state waters.

It was expected that A/T rich dinucleotide steps would have longer residence times with AA/TT giving the longest timescales as they are known to have a spine of hydration. While on the other hand, CG/GC combinations would give the shorter time scales. This was broadly found to be true but interestingly, the dinucleotide steps which were a combination of both also showed considerably long timescales at times comparable to pure AA/TT tracts.

3.2 Orientational Correlation Function. Orientational correlation function was calculated times for major and minor groove components for all 10 dinucleotide steps across all 20 DNA sequences. A typical OCF in major and minor groove is shown in Fig. 12. Similar to the residence time calculation, we have taken 0.35 nm as the cutoff for selecting water molecules in the first solvation shell. The orientational correlation function is a measure of average rotational dynamics of water molecules in the first solvation shell.

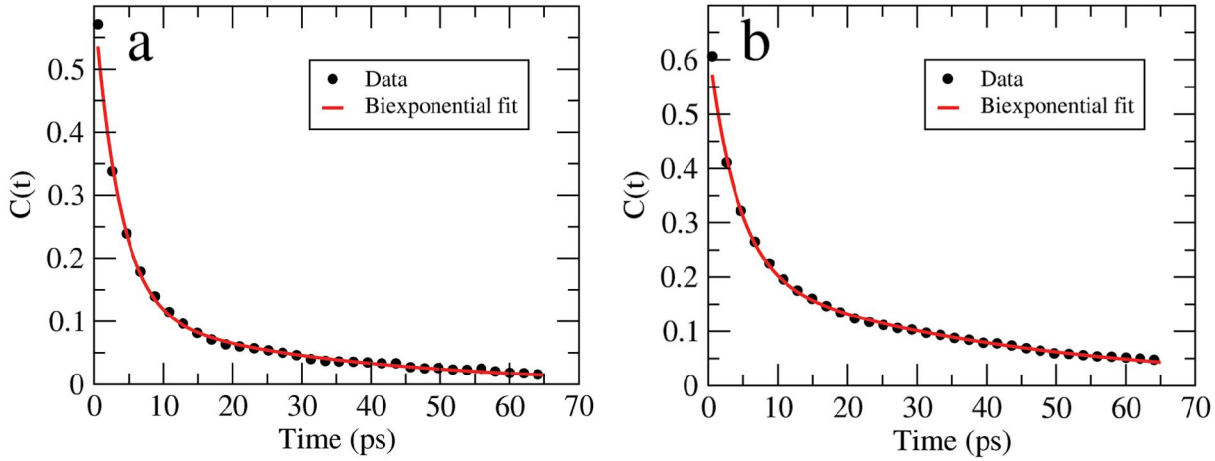

Figure 12: Typical orientational correlation plots for groove portions of a dinucleotide step. (a) Major groove (b) Minor groove

The orientational correlation function de-correlates slowly than bulk water as water molecules are held by hydrogen bonds with the electronegative atoms of the grooves. Also the shape of the grooves reduces the volume accessible for water to reorient, especially in case of minor groove where the groove is deep and narrow.

The initial 500fs of the orientational relaxation was not considered as this corresponds to librational motions, which is an ultrafast non-exponential phenomenon and the nature of librational relaxation remains unchanged on changing of solute hence it is of not of much interest to study in the context of distribution of timescales that we focus here. The initial 500 fs of librational contribution was discarded and the remaining dataset was

fitted again with a bi-exponential fit (Eq. 5). A scatter plot of the orientational timescales obtained was plotted to get an intuition of the spread of timescales (Fig. 13). Also, a histogram of orientational timescales was plotted (Fig. 14).

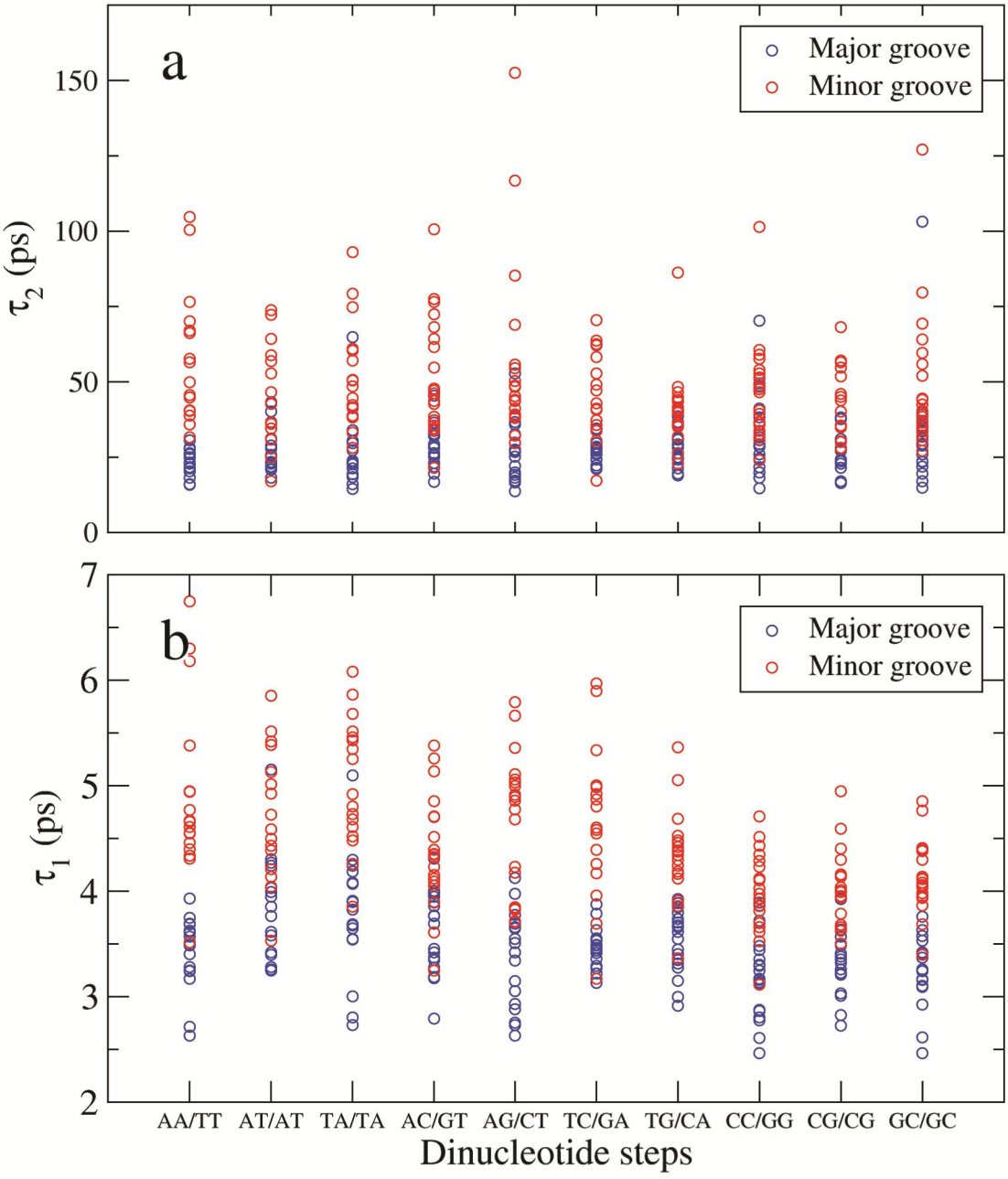

Figure 13: Scatter plot of orientational timescales obtained for every dinucleotide step. (a) Slow residence timescales (τ_1) (b) Fast residence timescales (τ_2).

The difference in the scatter of the fast and slow timescales is more pronounced here as compared to residence time scatter. Here though unlike residence time, there is no evident difference between the scatter of A/T dominated or C/G dominated dinucleotide steps. The heterogeneity in the reorientation times around DNA springs mostly from minor groove waters.

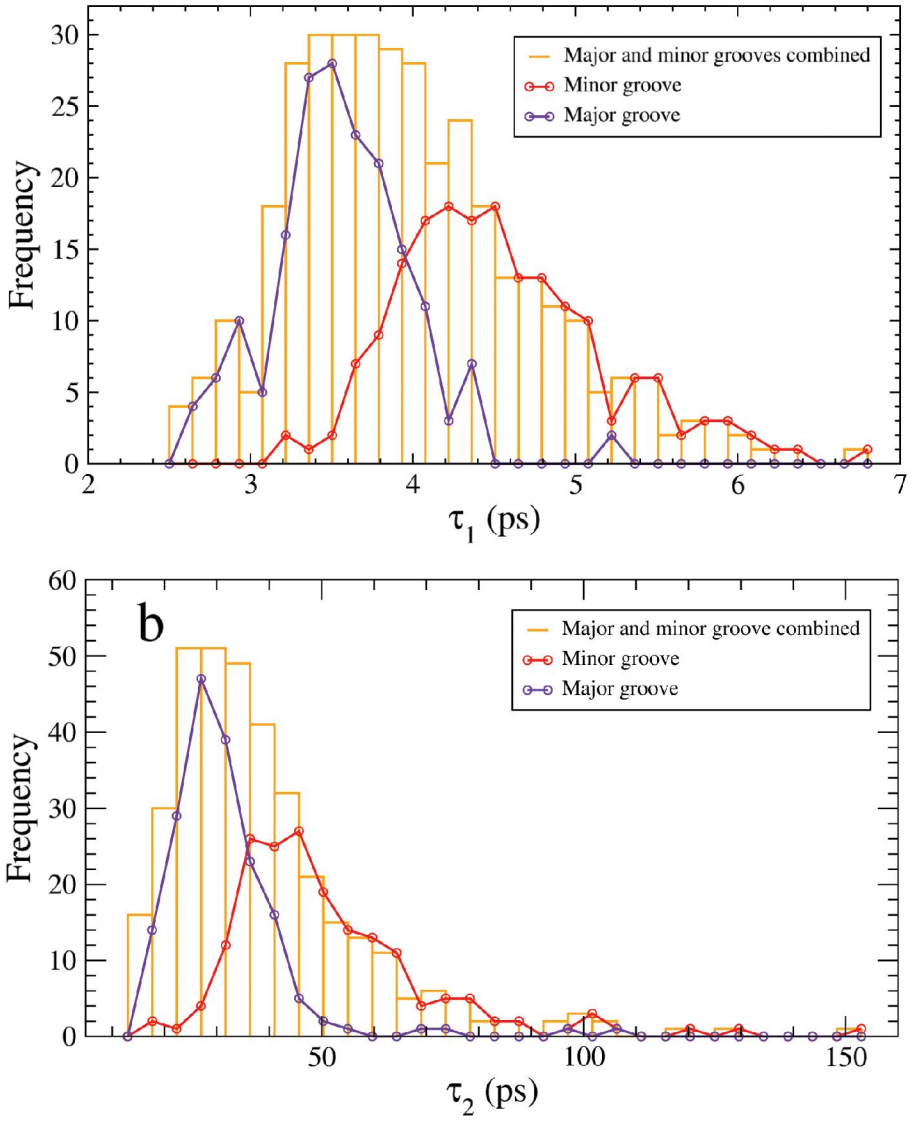

Figure 14: Distribution of orientational timescales. (a) fast timescales(τ_1) (b) slow timescales(τ_2).

The two timescales namely, the slow and the fast, again as stated before originate due to the presence of two types of water molecules, the free and bound water molecules. These affect the average reorientation times in the same way as that for residence times. The deep-seated bound water molecules, which are hydrogen bonded to electronegative atoms in the grooves of DNA, are responsible for slow re-orientational times. The free waters are the ones, which reside in first solvation shell but are not hydrogen bonded to electronegative atoms in grooves and have a behavior similar to bulk water. The reorientation time scale for a group of water molecules gives us information about the rotational mobility.

Here, again like the residence times distribution the distribution of both slow and fast reorientation times, the major groove distribution looks like a tall and narrow Gaussian distribution with a short tail which is although longer than one seen for respective residence time distribution. The minor groove distribution similarly resembles a short and wide Gaussian followed by a tail. Overall, the distribution of timescales in the first solvation shell follows a Gaussian distribution with a power law like tail.

3.3 Velocity Autocorrelation Function. The velocity autocorrelation of water molecules in the first hydration shell of the residue, on integration gives diffusion coefficient of the molecules. This helps us in understanding the translational dynamics of water molecules of water molecules.

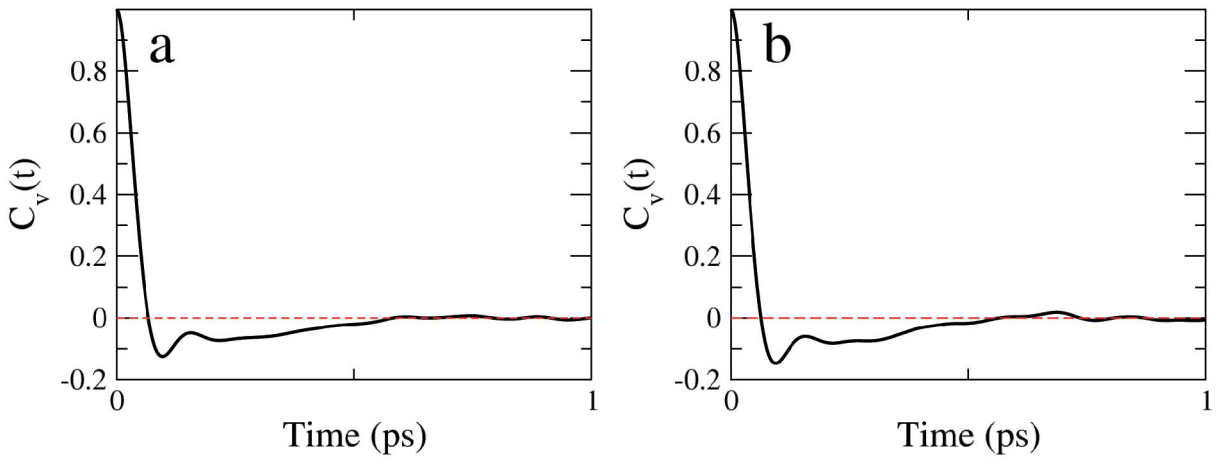

Figure 15: Typical VACF plots for groove portions of a dinucleotide step. (a) Major groove (b) Minor groove

The velocity autocorrelation plots for waters in first solvation shell of DNA residues were calculated with a cutoff of 0.35 nm for first solvation shell waters, a typical figure is shown in Fig. 15. These were compared to bulk water VACF plots. By general inspection of the plots, one can say that they have a different nature. The first trough for both occur at about same time but velocity autocorrelation value, C(t) for the DNA systems is lesser than that for bulk water by an order of magnitude. Further, the VACF for bulk water goes above 0 while the VACF for DNA system never goes above 0.

Integration of the obtained 360 velocity autocorrelation functions was done using simple trapezoidal rule. A scatter plot of the diffusion coefficients obtained was plotted in Fig. 16. This gives a sense of how the diffusion coefficients are distributed across the DNA.

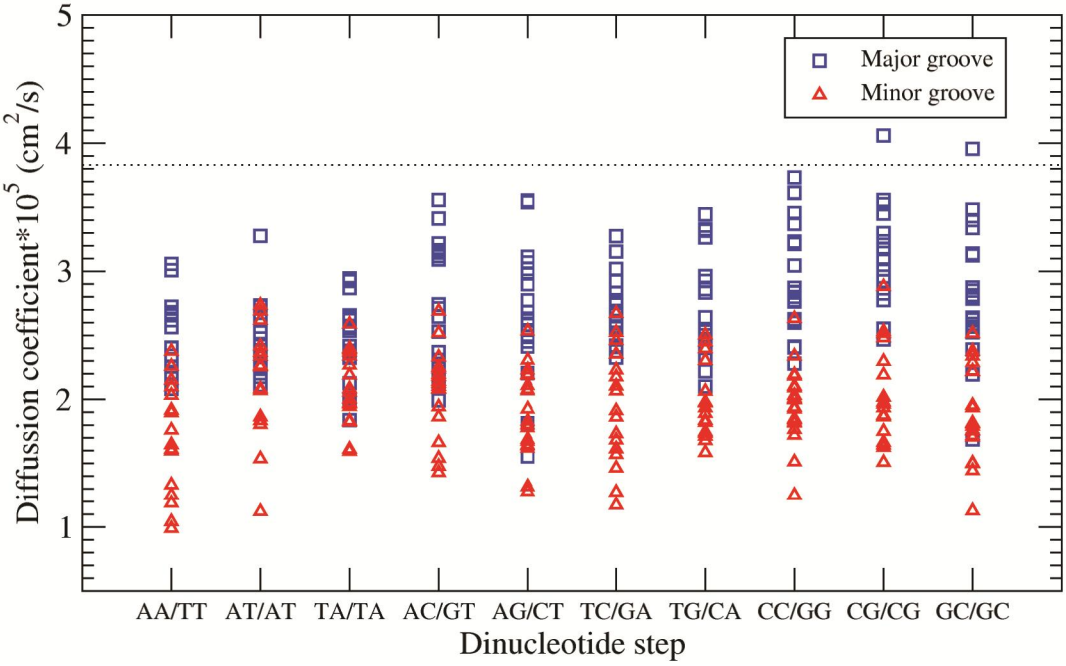

Figure 16: Scatter plot of diffusion coefficients obtained for every dinucleotide step.

By looking at the scatter plot, one can see that the diffusion coefficient, D is generally higher for major groove than the minor groove. Also, on average the diffusion coefficient is higher for C/G rich dinucleotide steps and it is lower for A/T rich steps. One can infer from the diffusion coefficients scatter plot that water molecules in the first hydration shell of the DNA are translationally more constrained near A/T rich sequences and have

more translational freedom around CG rich regions. This result was expected as A/T make stronger hydrogen bonds when compared to C/G. Also, the reason for minor groove waters having lower D than those in major groove can be easily explained by the presence the famous spine of hydration in minor groove. Frequency distribution of diffusion coefficients in the grooves was plotted to observe the heterogeneity of translational diffusive motion in the first solvation shell of DNA (Fig. 17).

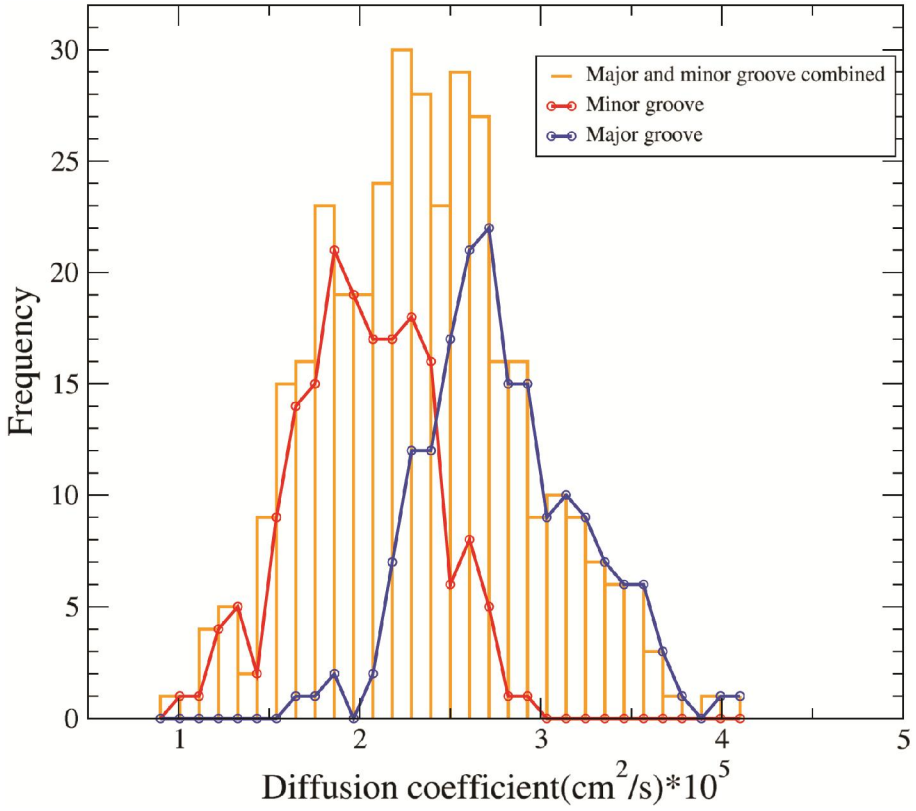

Figure 17: Distribution of diffusion coefficients

The distribution of the diffusion coefficients does not have the same features like that for orientational correlation function or residence time. Here, the distribution for both, the minor and major grooves seem to obey a Gaussian distribution with little or no hint of a power-law like tail. Also interestingly, the distributions for the major and minor grooves have similar heights and same widths, which is unlike the distributions we have encountered before. This change in the trend could be due to the fact that unlike residence time and reorientation times, which stem from the property of how slow or fast does the temporal correlation decays whereas diffusion coefficient is the result of

integration of the VACF plot and hence does not possess any temporal information. Thus, though it doesn't give us much information of temporal fate of translational relaxation of water molecules in the first solvation shell, but it does help us know how a water molecule near a particular residue of DNA, would behave for short stretches of time.

3.4 Discussion on the Distribution of Various Dynamical Quantities. The distributions for the orientational correlation, residence times and diffusion constants give us a clear picture of the heterogeneity in dynamical properties of water molecules in the first solvation shell of DNA. From the various timescales obtained for water molecules in the first solvation shell of DNA, one can gauge why there is a difference in the values reported by experiments which probe solvation shell water molecules of DNA. There has been an understanding from long time that water in the first solvation shell of AT rich sequences is the slowest and water near GC rich sequences is the fastest, which though true, does not paint the whole picture of water dynamics in the first hydration shell of DNA. One needs to realize that the distribution is heterogeneous and there is a considerable deviation of water behavior for a similar site (in our case, a dinucleotide step). Such a behavior is mostly but not always a consequence of the influence of flanking nucleotides. Also at times dinucleotides like AC/GT or TG/CA exhibit very high slowdowns or at times, surprisingly low slowdowns. Thus, it is difficult to predict the water dynamics in the solvation shell of a DNA just by looking at its sequence. The portion of the distribution corresponding to the tail is predominantly due to minor groove and these are the slowest waters in the first solvation shell.

The cause for the heterogeneity in the distribution could arise from two factors, one, the chemistry of the groove atoms and two, structure or arrangement of the groove atoms. The chemistry of the constituent groove atoms which form hydrogen bonds with water molecules could be an important factor as stronger the nature hydrogen of hydrogen bond, the greater is its lifetime and consequently lesser is its orientational freedom and mobility. On the other hand, the structure of the groove, its topology may lead to a reduction in volume accessible for a hydrogen bonded water molecule to rotate or vibrate which would eventually lead to a hydrogen bond with a lifetime longer than that

anticipated from its chemical nature. If the dynamics were totally due to chemistry, then there wouldn't be any tail in the distribution. The major and minor groove distributions would have been two distinct Gaussian distributions with different means but almost similar spread. So it looks like the causative for slowdown of waters in the grooves is a combination of both.

3.5 Water reorientation mechanism. Physically, water reorientation is rotational relaxation of water molecules. The mechanism of water reorientation in bulk water was reported by Laage et al.[50] They suggested a pathway in which a water molecule breaks a hydrogen bong with an over coordinated first shell neighbor and consequently forms a hydrogen bond with an incoming second shell neighbor. Such a reorientation proceeded through a large amplitude angular jump and also diffusion limited frame re-orientation [52]. We have tried to observe the water reorientation mechanism for the waters in the first shell of DNA. Here, the donors are exclusively hydrogen atoms of water molecules in the first shell while the hydrogen bond acceptors are water oxygens in the first shell of DNA and the electronegative (O, N) atoms of the DNA. We can consider this reorientation process as a "switch". A switch involves one donor and two acceptor molecules. We can model this switch process as the following reaction:

$$O_a\text{---}O^* \quad O_b \longrightarrow [O_a\text{---}O^*\text{---}O_b] \longrightarrow \quad O_a \quad O^*\text{---}O_b \qquad \text{(Scheme 1)}$$

where, O* represents the donor atom, O_a the former acceptor and O_b the latter acceptor. A schematic representation of the reorientation mechanism near DNA is depicted (Fig. 18) involving a water molecule switching between two electronegative atoms of DNA.

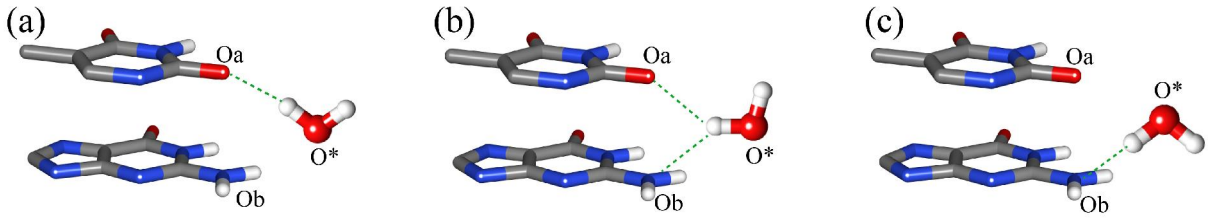

Figure 18: Schematic representation of a switch from one base pair atom to another base pair atom. (a) Water molecule hydrogen bonded to Oa (b) Transition state for the "switch" reaction (c) Water molecule hydrogen bonded to Ob

The following reaction coordinates were monitored for the "switch" reaction: R_{O^*Oa}: Distance between O* and Oa; R_{O^*Ob}: Distance between O* and Ob; Θ : Angle between projection of O*H* vector on OaO*Ob plane and angle bisector of OaO*Ob. The investigation for jump reorientation mechanism was first done for bulk water (Fig.19). The results for bulk water reorientation matched exactly with the results reported by Laage *et al* [50]. Then, we attempted to observe the mechanism of switching in the first solvation shell of DNA. We have extracted all the switch instances with water molecule in the first solvation shell as a hydrogen bond donor and all the possible hydrogen bond acceptors atoms (electronegative atoms of DNA along with water molecules in first solvation shell). Such an analysis was done various components of DNA, namely, minor groove, major groove, and phosphates (Fig. 20).

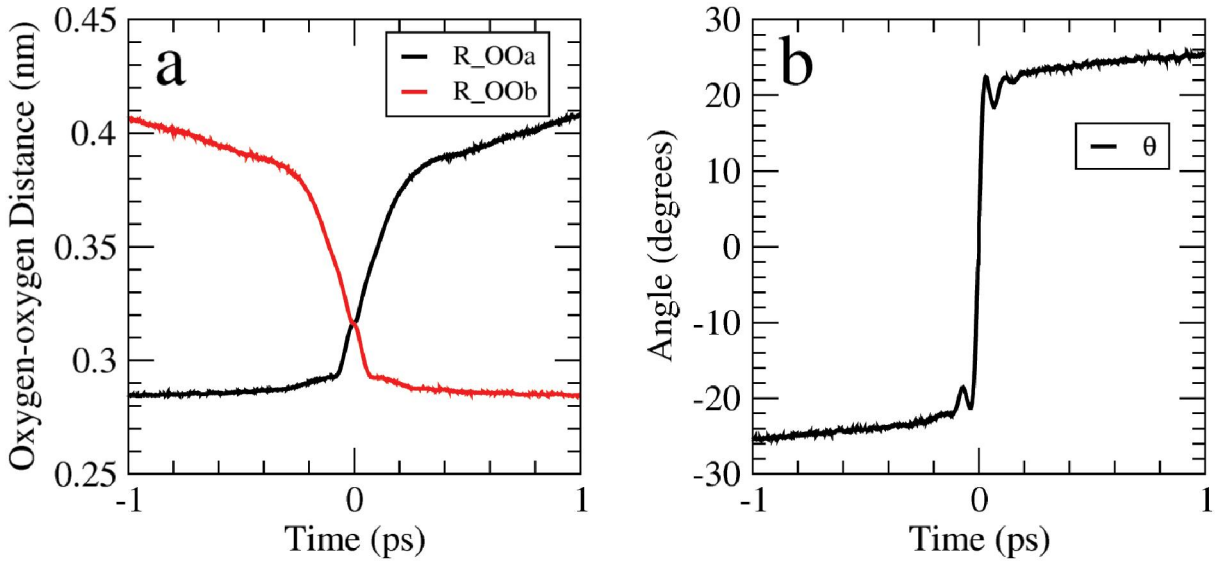

Figure 19: Time evolution of reaction coordinates for the switch reaction in bulk water (a) Distance between acceptors and donor oxygen atoms. (b) Angle between projection the of donor OH bond on the plane formed by acceptors and donor and the angle bisector of donor acceptor vectors.

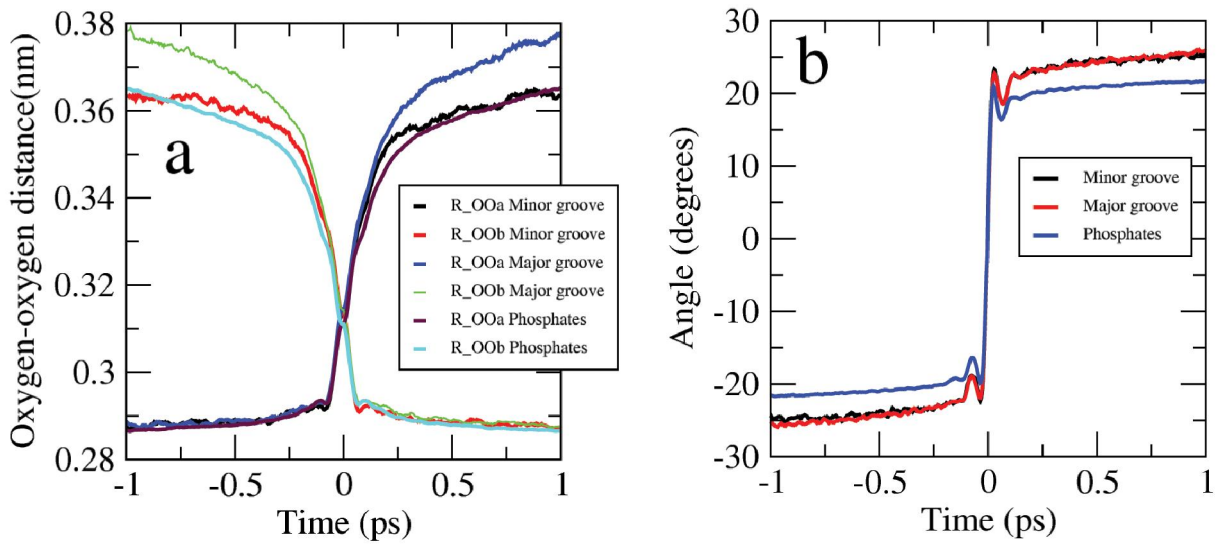

Figure 20: Time evolution of reaction coordinates for the switch reaction for DNA in water system (a) Distance between acceptors and donor oxygen atoms. (b) Angle between projection the of donor OH bond on the plane formed by acceptors and donor and the angle bisector of donor acceptor vectors.

Distance between oxygens is a slow coordinate whereas θ is a fast coordinate of the reaction. It helps to understand the mechanism of switch as it is highly sensitive around time of switch (t=0). On inspection of the of reaction coordinate plots one can understand that the only appreciable difference is found for phosphates which is obvious as the phosphate groups have multiple oxygens in close proximity and hence, generally a water molecule keeps switching between the oxygens of the same phosphate group. One general observation that can be made when comparing with bulk water plots is that the change in the angle θ is lesser for DNA, which again is expected due to the easy availability of acceptor sites on the surface DNA. The mechanism of water reorientation was not found to be quite different from that of bulk and hence, it can be said that water molecules in the grooves of DNA have similar mechanism for reorientation as bulk water.

4. Conclusions

We have calculated various water dynamical properties (residence time, orientational relation, diffusion coefficient) around the local DNA environment via various dinucleotide steps. The most important observation is that all the different properties show a distribution of timescales, rather than a particular fixed value, which explains the ambiguity in water dynamical timescales obtained from different experiments. Both the residence time and the orientational relaxation show biexpoential behavior with a short and long relaxation time. In general, water dynamics in the minor groove is slower than that of the major groove. However, decomposing the distribution to individual dinucleotide steps show that even for the same dinucleotide step, there is a significant scattering of timescales for both the short and long time. Going from A/T to G/C the scatter decreases, indicating that the chemical nature of the dinucletide steps are important. For the mixed sequence, the timescales are somewhat unpredictable and needs further investigation to find their origin. However, the fact that each of the dinucleotide step differs in the timescale indicate either the role of the flanking sequence or the effect of topology which in turn is an effect of local sequence variability. The important observation stems from this study is the role of topology and chemistry. This study thus points towards an intriguing chemical physical aspects of various recognition processes that are mediated by water, where the variability in water dynamical timescale originating from the variablity of DNA sequence affect indirectly the recognition process itself. Therefore, this study calls for further investigation on the source of variability in the context of DNA recognition processes and work along these lines is under progress.

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