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**Comparison of global and local motional properties of human telomere repeat binding factor (hTRF2) in vacuum and water**

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**ABSTRACT**

*A fundamental appreciation for how biological macromolecules work requires knowledge of structure and its dynamics. Molecular dynamics simulation provides links between structure and dynamics by enabling the exploration of the conformational energy landscape accessible to protein molecules. In this perspective we illustrate the application of molecular dynamics simulations to biology by describing the conformational changes of Human Telomere Repeat Binding factor 2 (hTRF2). hTRF2 is a sequence specific DNA-binding protein. The progress of the simulation was monitored by calculating several structural parameters over time in both the vacuum and water condition. The result shows some huge amount of fluctuation in the region of N-terminal end of the hTRF2 that give us some new information about the structural changes of the global protein.*

**Keywords:** Telomere, Helix Turn Helix motif, MD simulation, GROMACS, DNA-protein interaction.

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**INTRODUCTION**

Telomeres are repetitive DNA sequences located at the termini of linear chromosomes of most eukaryotic organisms, and a few prokaryotes. The tips of the DNA molecule - **telomeres** contain a kind of chain of repeating pairs of enzymes, telomerase compensate for incomplete semi-conservative DNA replication at chromosomal ends. The protection against Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ) constitutes the essential “capping” role of telomeres that distinguishes them from DNA double-strand breaks (DSBs) [1].

All known eukaryotic telomeres consist of simple repeated sequences of G- and C-rich complementary strands, with the general structure (T or A)<sub>m</sub>(G)<sub>n</sub> [2].

Structural information on telomeric proteins shows that, despite a lack of extensive amino-acid sequence conservation, telomeric DNA recognition occurs via conserved DNA-binding domains [3]. The Telomere Repeat Binding factor TRF2 is responsible for interaction with Rap1 and Mre11 complex [4]. TRF2 contains the following functional domains- (i) a central TRF-homology domain, TRFH (ii) a C-terminal DNA binding domain and (iii) an N-terminal domain that is basic in nature. TRF2 protein has a Myb-like helix-turn-helix domain in their carboxy terminus and a central conserved domain (TRFH) that includes sequences responsible for the formation of homodimers [5].

The solution structures of the DNA binding domain of hTRF2 consist of 63 amino acids [6] with a methionine residue at its N terminus. The DNA binding domain consists of the well known helix turn helix motif where the helix 3 interacts with the major groove of the DNA and the N terminal with the minor groove of the DNA. The nine amino acids of hTRF2 are responsible for the DNA binding action of hTRF2 [7]. The structures contain three helices, **helix 1** from Val452 to Tyr465, **helix 2** from Trp470 to Asn476, and **helix 3** from Ala484 to Arg496.

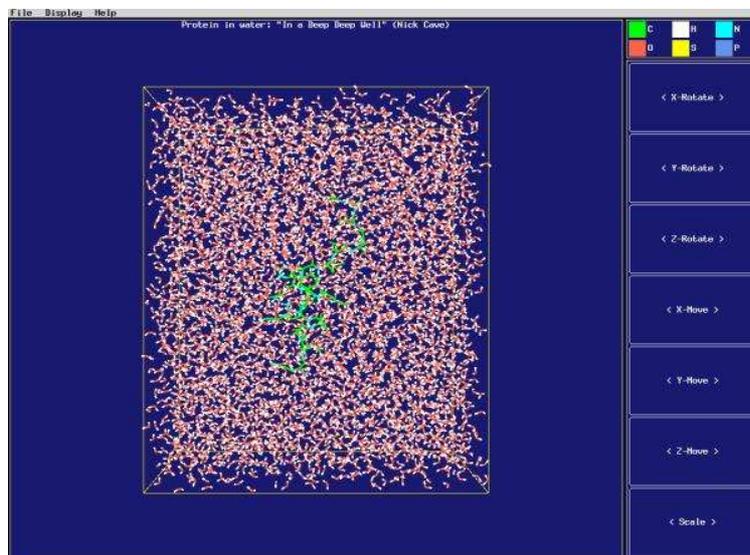
The dynamic properties of proteins are now well known to play important roles in protein function. Many different aspects of protein function can be affected by protein dynamics. For example, protein–protein recognition, protein–DNA interactions [8] and enzyme–substrate binding and enzyme activity are all determined, by the conformational flexibility of the protein backbone as well as specific side chains. It is, therefore important to characterize not only the structure of a protein but also its dynamic properties as well. Here in this study we compare the global protein and as well as the local parts of the protein in vacuum and in water to observe the dependency of motion of whole protein on local motions.

## EXPERIMENTAL SECTION

The use of computer simulations to probe protein motions, using existing structural information, is, therefore, proving extremely fruitful. Trajectories generated from molecular dynamics (MD) simulations provide a means to identify and study motions crucial for protein function. Separating functionally important motions from random thermal fluctuations is a major challenge in analyzing MD trajectories.

In this study, we use MD simulations in conjunction with an essential dynamics analysis to look for conserved dynamics of the protein. All simulations were carried out using the GROMOS 96 Force Field [9] within the GROMACS software package [10]. Taking the NMR structures of hTRF2 (PDB ID: 1VF9) in a cubic box with a 4.0 Å edge length. The simulation was run at a constant temperature of 300 K and a constant pressure of 1 atm. The temperature and pressure were regulated by weak coupling to an external bath. An electrostatics interaction as Van der Waal and coulombic, cut-off was dealt with using a radius of 10 Å. First, the structure was energy minimized using steepest descent for 10,000 steps and same condition was set for conjugant gradient. Second for the solvate condition the “SPC” water model (spc216.gro file) was used to fill up the box. The output configuration contains 68055 no. of solvent molecules (water) [Fig 1]. But for vacuum condition this step was not done. Then a position restrained MD run was carried out which holds the protein atoms fixed and finally MD simulation was done for 1000 picoseconds and the total no. of steps required was 500000 (total nsteps). The atoms in the system were given initial velocities according to a Maxwellian distribution. The system was

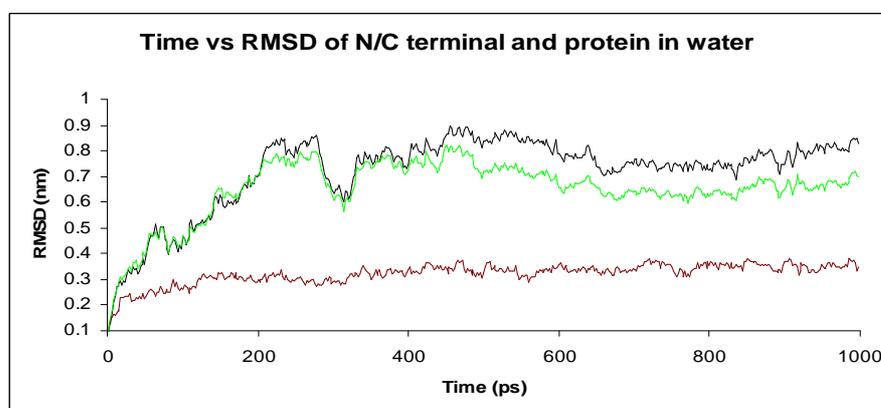
allowed to evolve according to Newton's equations of motion, with the equations being integrated with time step of 0.002 picoseconds by the known algorithms [11]. The progress of the simulation was monitored by calculating several structural parameters over time: C-alpha R.M.S.D, chain compactness is also probed by monitoring the radius of gyration for the protein over time, (Rg) and C-alpha RMSF per residue for the native-state simulations only. Each of these structural properties was calculated within GROMACS.

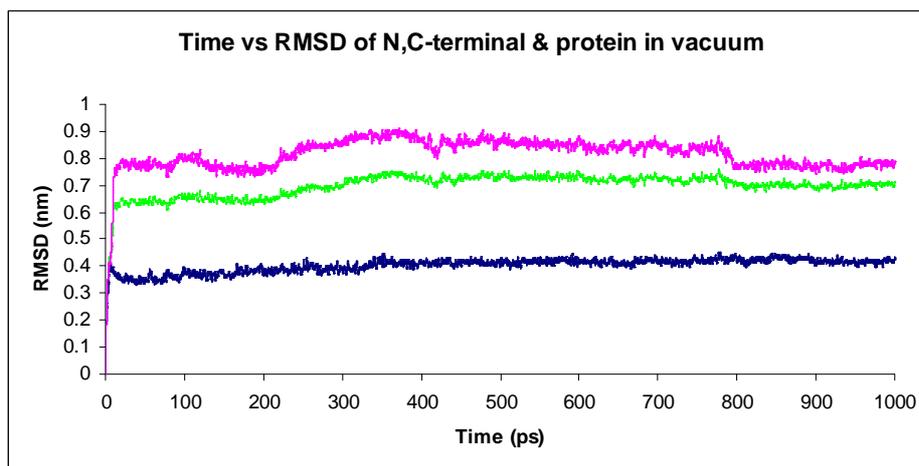


**Fig 1:** A cubic box filled up with water molecule (in red) and the hTRF2 protein (in green) is solvated by these water molecules

## RESULTS AND DISCUSSION

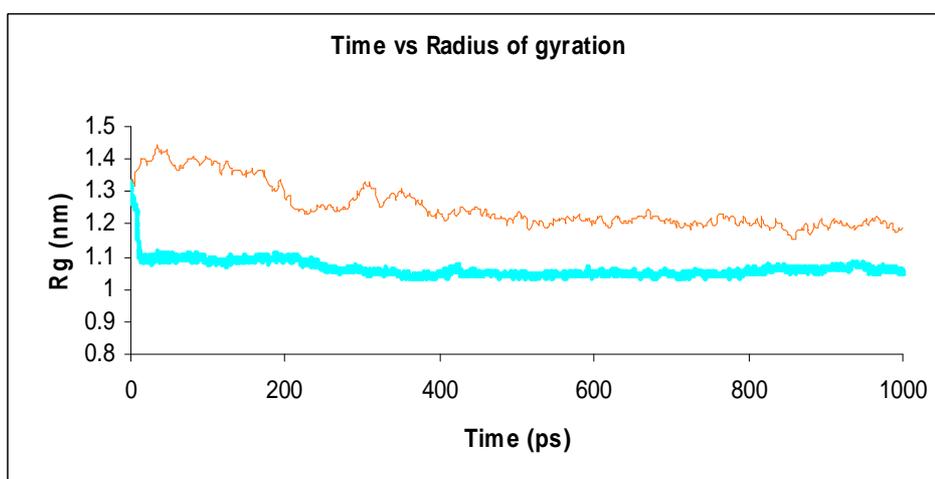
The backbone dynamic was observed by monitoring average C- $\alpha$  root mean square deviation (RMSD) over time, and individual C- $\alpha$  root mean square fluctuations (RMSF) per residue. Chain compactness and to check the stability of the simulation, radius of gyration is also monitored for the protein over time. The plot of C- $\alpha$  RMSD versus time [Fig 2(A) & 2(B)] clearly shows that there is an initial spike, after which the system appears to reach an equilibrium value which does not change with time and it is very interesting to see that the nature of curve for the N terminal [12] (black in water and pink in vacuum) and the protein (green in water and vacuum) is near about similar.





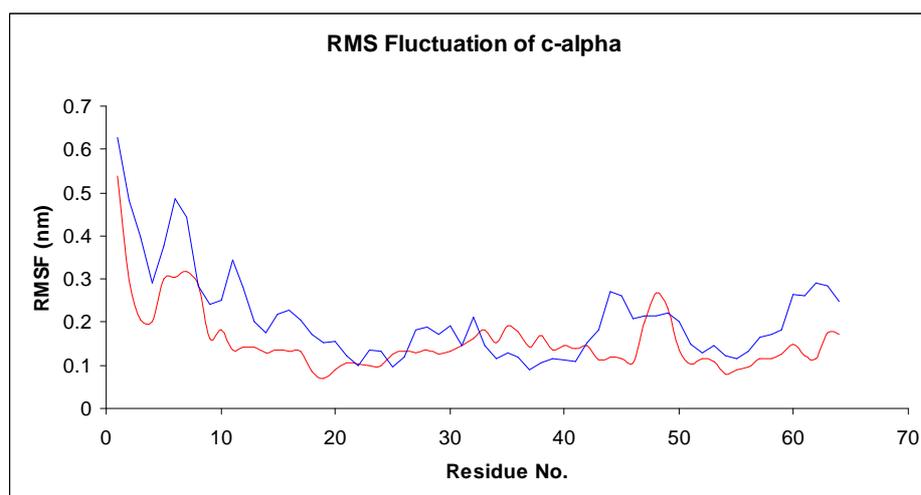
**Fig 2: (A) showing the RMSD vs Time plot of the N, C-terminal and of the full protein in water condition. (B) Same parameters have chosen in the second plot but in vacuum condition**

As there is a huge fluctuation in the first ~350 ps in solvent the average RMSD for N terminal and the protein is between 0.7-0.8 nm but for the C terminal in both the cases the average RMSD is much less than the others. The Rg remains constant [Fig 3] throughout the simulation for hTRF2 in the vacuum condition (curve in blue colour) in the range of 1.1nm. Again the plot gave the view that in water condition (curve in red colour) the protein in the first time slot up to 400ps the Rg gave the value near 1.35nm but after that time period it stabilized at 1.2 nm range.



**Fig 3: Radius of gyration, distance of the objects' parts from either its center of gravity or an axis are shown here of TRF2 in vacuum (blue) and solvent (red) condition**

The flexibility of a protein is also often revealed by looking at the root mean-square fluctuation (RMSF) of each residue from its time-averaged position. The N-terminal is a free arm & the C-terminal is globular in nature and hence make many hydrophobic interactions so the fluctuation of N terminal residues shows more flexibility than C terminal from RMSF curve [Fig 4]. As the N terminal end is free so during the dynamics run the fluctuation in the RMSF curve also shows the maximum peak.



**Fig 4:** RMSF of C-alpha atoms with respect to their average position over the entire time simulation in solvent (red curve) and vacuum (blue curve) condition

Telomere Repeat binding factor2 (TRF2) is an essential sequence specific DNA binding transcription factor where the DNA binding domain constitutes the Helix Turn Helix motif with a free N terminal end [13]. An increasingly detailed and complete picture of telomeric DNA sequence organization and structural variation is essential for understanding and tracking allele-specific subterminal and subtelomeric features critical for human biology. This N terminal end interacts with the minor groove of the DNA to hold the protein firmly during the transcription process. The dynamics simulation was carried under the water and vacuum condition but surprisingly all the above figures indicate that the curve nature of the full protein is near about same as the curve nature of the N terminal end. Comparing the global and the local motions of hTRF2 it is proved that the local N terminal fluctuation changes the full protein fluctuation nature. But also the middle portion of the curves which is stable and same for local and global dynamics give a hint that the helix portion that interacts with the DNA major groove does not change their structure. This reflects that the binding site residues show less mobile nature that confirms its rigidity and is useful for modeling drugs as it may be represented as binding pocket for inhibitors [14]. As the telomere end is also very crucial for human aging and the hTRF2 help in telomere length regulation [15] so may be the motional property of hTRF2, can in future help in predicting the much known problems of human world.

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