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# 3D Structure Modeling of Human Telomere Repeat Binding Factor 2 and DNA-Protein Docking Studies

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***Authors' contributions***

*The following work was evenly distributed among all authors. Author KM designed the study and carried out the main work frame. Authors DMP and ASV help in analyzing the results and arranging the result and discussion part. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** Human telomere repeat binding factor (hTRF2) is a double stranded telomere binding protein that plays key role in protecting the chromosome ends and a necessary building block of telomere structure maintenance. The aim of the present study was to focus on the modeling of 3D structure of hTRF2 (500 residues long) and its interaction studies with DNA *in silico*.
**Study Design:** The overall work was designed in different steps starting with the modeling of the concerned protein, its physiochemical properties study, modeling of 3D-DNA with specific length and varying bend angle, docking studies of modeled DNA and hTRF2 protein.
**Place and Duration of Study:** Bioinformatics Lab, Department of Biotechnology, Birla Institute of Technology, Mesra, India. November 2012- July 2013.
**Methodology:** 3D structure of hTRF2 was modeled through I-TASSER method. The modeled structure was verified by 5ns of simulation run in solvent (water) condition and also evaluated with different bioinformatics tools. Physiochemical properties were calculated through CLC Protein Workbench. DNA 3D structure was modeled with the conserved nucleotide sequence motif, TTAGGG with varying bend angles of 0° to 60°. The DNA-protein docking studies were carried out through HADDOCK easy interface for

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each bend angle.
**Results:** The best model was selected depending on minimum RMSD value and C-Score and the Stereochemical quality of that model was verified with different tools, as the Molprobity score (>1) of hTRF2 was predicted 4.2 and Ramachandra favored residue was 80.56%. The selected model protein and DNA structure was docked and among all the docking results the best orientation of DNA and hTRF2 was at 60° DNA bend angle with lowest RMSD and maximum Z-value. The amino acids which are directly involved in the interaction were also selected.
**Conclusion:** In future further study will be planned with further bend angle for getting better information on DNA-protein interactions. *In silico* studies will also be helpful for the researchers to study the complex structure *in vitro*.

*Keywords: DNA-protein docking; ab initio modeling; telomere binding protein; transcription factors.*

## 1. INTRODUCTION

A wide number of proteins, involved [1] in the process of converting or transcribing, DNA into RNA are referred as transcription factors (TFs) [2,3]. One distinct feature of transcription factors is that they have DNA binding domains (DBD) that gives them the ability to bind to a specific DNA sequence, well known as enhancer or promoter regions [4]. Some TFs bind to a DNA promoter [5] sequence near the transcription start site and help forming the transcription initiation complex [6]. Other transcription factors bind to regulatory sequences, such as enhancer sequences [7], and can either stimulate or repress transcription of the related gene [8].

Telomere binding proteins (TBP) recognize the short tandem repeats of telomere DNA [9] to preserve the stability of chromosome structure. These types of proteins help telomerase, ensuring the appropriate length of DNA [10,11] during the information flow from DNA to protein. Human telomere repeat binding factor 2 (hTRF2) is a very well known transcription factor showing sequence homology with hTRF1 and binds telomeric DNA consists of simple repeated sequences of G- and C-rich complementary strands, with the general structure $(T \text{ or } A)_m(G)_n$ [12]. hTRF2 interacts with Rap1 [13] and the Mre11 complex composed of Mre11, Rad 50, and Nbs1 [14] and helps the RNA polymerase at the endpoint of DNA strand for protection and maintenance [15]. Structural information on telomeric proteins shows that, despite a lack of extensive amino-acid sequence conservation, telomeric DNA recognition occurs via conserved DBDs [16]. Human TRF2 (hTRF2) is mainly classified as a *Myb*-type DNA binding protein. It comprised of a basic N terminus, a central homodimer specific conserved domain (TRFH) and a C terminus [17]. The total length of hTRF2 is 500 residues and the DNA binding domain (DBD) can be found in C terminus. The DBD is composed of a helix turn helix (HTH) motif of 64 residues. Helix 2 and Helix 3 are connected with a sharp turn to form the HTH motif [18]. Helix 3 is known as recognition helix which mainly interacts with the major groove of the DNA during the DNA-protein interaction. The positive residues in the helix 3 region can make a good bonded and non-bonded interaction with negatively charged nucleotides of DNA [19].

Telomere binding proteins with HTH motif [20] are crucial in telomeric length regulation, associated with aging problem. Several studies in recent years [21] already demonstrated the involvement of DNA binding domains of different proteins like: RTBP1 [22], NgTRF1 [23],

hTRF1 [24] and Rap1 [25] in telomeric DNA end protection. But till now full protein structure is not predicted through X-ray crystallography or computationally. But it is essential to study the same interactions *in silico* as a replica of the *in vivo* studies. Different scientific advancement can help in this regard with 3D modeling of protein and DNA, structure validations with accurate force field and long simulation runs, cracking the docking studies. Molecular dynamics (MD) is the study of the motions of atoms within a desired time scale of several nanoseconds. Recently in Balu, et al. [26], article MD studies were conducted on the structures of NSP3 and p130C to measure the changes after incorporating some mutations [26]. Kumar and Purohit [26] incorporatemutation in AKT1 PH domain and compare the stability loss in both the structures (native and mutant). But some drifts were observed in the mutant due to rapid conformational changes and maybe these changes can alter the pathological effects [27]. Similar work [28] was also conducted on lamin A/C protein, involved in the mechanism of laminopathy.

So, this was an attempt to model the full protein, validate the structure and to study the DNA-protein complex structure.To characterize the sequence and structure of these proteins individually the present available bioinformatics tools and software's helps us to complete the analysis in a very short time. Here we focus on the protein structure modeling and validation, characterization of the modeled protein and its interaction studies with DNA through docking.

## 2. MATERIALS AND METHODS

### 2.1 3D Structure Modeling and Binding Site Prediction of hTRF2 Protein

Normal homology modeling was failed to construct the 3D structure of the full proteins due to the unavailability of the suitable template sequence for query sequence (hTRF2). The I-TASSER server modeled the protein structure through threading approach which states about the stitching of small fragments of proteins after modeling. For modeling I-TASSER searches the possible templates in PDB first (with a pair-wise sequence identity cut-off of 70%) and if template is not found then it runs the *ab initio* method to predict the 3D structure of protein [29]. 3D models of proteins were build based on multiple-threading alignments by LOMETS and iterative TASSER assembly simulations [30,31]. The 3D structure of hTRF2 verified using different online bioinformatics tools (Molprobity, VERIFY3D and PROCHECK). The active residues of the proteins within the HTH motif region were identified through ScanProsite [32] available at http://prosite.expasy.org/scanprosite/.

### 2.2 Energy Minimization

To check the stability of the modeled protein structure, energy minimization was also carried over through GROMACS. GROMOS 96 Force Field [33,34] within the GROMACS software package was applied which is available at http://www.gromacs.org/ [35].Energy minimization was performed in two steps namely, steepest descent (steep) and conjugant gradient (cg) for 10,000nsteps each. The simulation was conducted for 5ns time slot in solvent (water) condition at a constant temperature of 300 K and a constant pressure of 1 atm and each component was coupled separately to an external bath using the Beredson coupling method [36].

## 2.3 Characterization of hTRF2 Modeled Protein

The different physicochemical properties of the protein were computed using CLC protein workbench (http://www.clcbio.com/index.php?id=27) which includes the following parameters; molecular weight, extinction co-efficient [37], half-life [38], theoretical isoelectric point, instability index, aliphatic index [39]. Kyte-Doolittle scale [40] and Eisenberg scale [41] were selected for the hydrophobicity calculation with a fixed window size (of an odd number) over the protein sequence. CYS_REC was used to locate "SS bond" between the pair of cystein residues (http://linux1.softberry.com/berry.phtml?topic=cys_rec&group=programs&subgroup=propt), if present.

## 2.4 3D-DNA Modeling

Template DNA 3D structure was modeled by 3D-DART web server (http://haddock.chem.uu.nl/enmr/services/3DDART/) [42]. The animal telomeric DNA template sequence was retrieved from telomeric database (http://telomerase.asu.edu/sequences.html). The length of designed template DNA sequence was 30 nucleotides with 6 repeats of the TTAGGG sequence. While modeling DNA, the bend angle was chosen within a long range of 0° to 60° with tilt of 5° as global changes. The output file generated for 3D-DNA structure was compatible for Haddock input.

## 2.5 DNA-Protein Docking Study

In the following study the easy interface of HADDOCK, available at HADDOCK web server (http://haddock.science.uu.nl/services/HADDOCK/haddockserver-easy.html) was employed. It is an information-driven flexible docking approach for the modeling of biomolecular complexes. HADDOCK [43] is different from other *ab initio* docking methods in the fact that it encodes information from identified or predicted protein interfaces in ambiguous interaction restraints (AIRs) to drive the docking process [44]. For the docking study two inputs were required, the DNA molecule and the protein molecule. The pre-requisite modeled structure of DNA was the first molecule. The full length of DNA structure was 30 nucleotides and the active residues were selected mainly; T15, T16, A17, G18, G19, T20, T21, A22, G23, G24, T25. Other residues were selected as passive residues. This condition was same for all the bend angles of animal DNA. The second molecule was the modeled protein structure (hTRF2). The identified active residues from Scan Prosite result were classified into three regions: helix3 region, N terminal+helix3 region, N+C terminal region (Table 1). Individually all three categories were applied as active residues during the docking studies. Other residues were selected as passive residues.

**Table 1. Description of three regions of active residues, length and their position in hTRF2 protein derived from ScanProsite result**

| | | |
|---|---|---|
| **helix3** | **Position** | 484-496 |
| | **Length** | 13 |
| | **Residue** | AVMIKDRWRTMKL |
| **N terminal + helix3** | **Position** | 438-450, 484-496 |
| | **Length** | 25 |
| | **Residue** | EDSTTNITKKQKWAVMIKDRWRTMKL |
| **N+C terminal** | **Position** | 438-450, 497-500 |
| | **Length** | 15 |
| | **Residue** | EDSTTNITKKQKWGMN |

## 3. RESULTS AND DISCUSSION

I-TASSER method predicted five models of hTRF2 protein structure with different C-score (Confidence score), TM score and RMSD for estimating the quality of predicted structure. The best model (model1) was selected depending on minimum RMSD value and C-Score (-0.85) (Fig.1.). C-score of higher value (Range: [5,2]) signifies a model with a high confidence and vice-versa. The other four models produce different values for the above parameters which are inferior to the selected model. Superimposition was also conducted for all four models with reference to selected model (model1) and the minimum RMSD was calculated (0.628A) with model1 and model4.The result also includes the secondary structure prediction, solvent accessible area, GO terms, binding site prediction with nucleic acid or any other ligands, top 10 maximum similar structures with the query and etc.

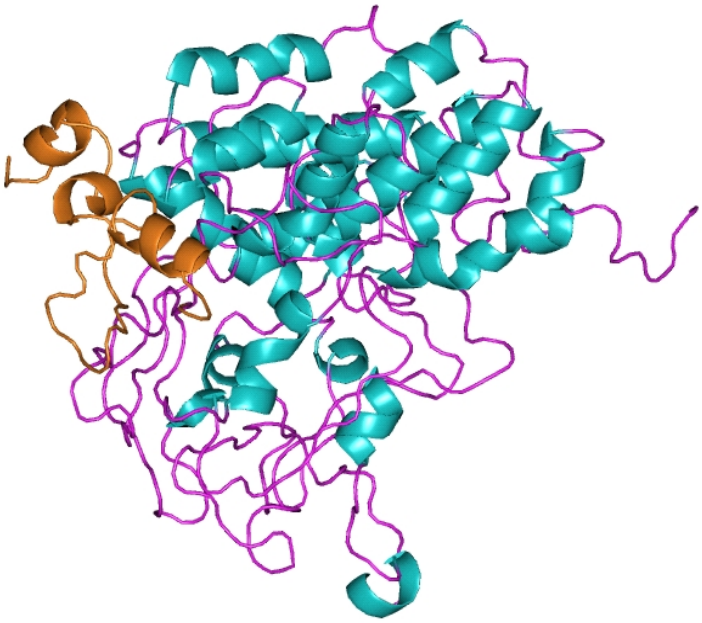


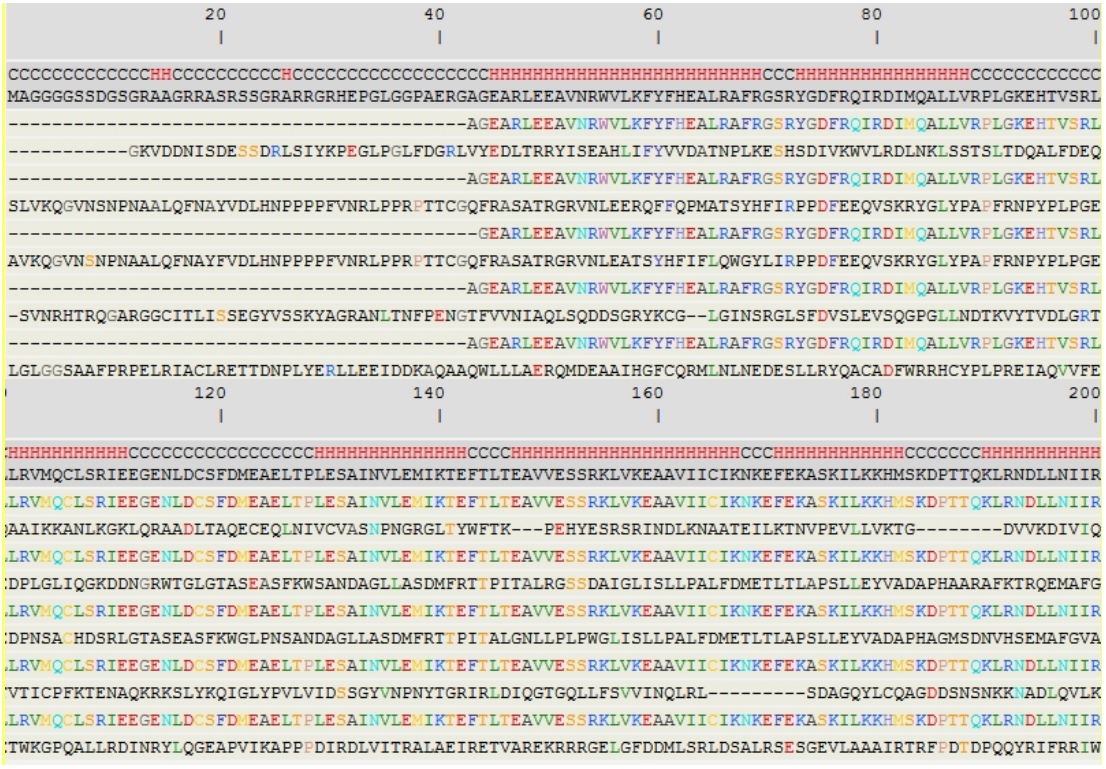
**Fig. 1. 3D structure of hTRF2 protein predicted by I-TASSER. The helixes are represented in cyan color, coils in magenta and DNA binding domain region in golden yellow.**

The I-TASSER method also shows best ten template structure with its PDB hit, normalized Z score of the threading alignment, percentage sequence identity of the whole template and in the threading aligned region, coverage of the threading alignment. The table (Table 2) also indicates that the query sequence is having maximum sequence alignment with two templates, 3cghA (0.84) and 3gzsA (0.85). The multiple sequence alignment (Fig. 2.) was also performed by I-TASSER method for the query sequence with the top ten template sequences for better understanding about the matching and mismatching portions. As the full protein sequence is having less matching with template than the DNA binding domain part which is situated in the C terminus.

**Table 2. Top 10 templates used by I-TASSER.**

| Rank | PDB hit | Identity1 | Identity2 | Cov. | Norm. Z score |
|---|---|---|---|---|---|
| 1. | 3bu8B | 1.00 | 0.41 | 0.41 | 2.19 |
| 2. | 3cghA | 0.10 | 0.19 | 0.84 | 1.62 |
| 3. | 3bu8A | 1.00 | 0.41 | 0.41 | 9.04 |
| 4. | 3gzsA | 0.10 | 0.20 | 0.85 | 1.59 |
| 5. | 1h6pA | 0.98 | 0.38 | 0.38 | 4.68 |
| 6. | 1h6pA | 0.97 | 0.38 | 0.38 | 2.22 |
| 7. | 3bu8A | 1.00 | 0.41 | 0.41 | 3.83 |
| 8. | 3bu8B | 1.00 | 0.41 | 0.41 | 6.73 |
| 9. | 3bqoA | 0.29 | 0.14 | 0.40 | 6.40 |
| 10. | 1h6pA | 0.98 | 0.38 | 0.38 | 4.41 |

** Ident1 is the percentage sequence identity of the templates in the threading aligned region with the query sequence; Ident2 is the percentage sequence identity of the whole template chains with query sequence; Cov. represents the coverage of the threading alignment; Norm. Z-score is the normalized Z-score of the threading alignments.*



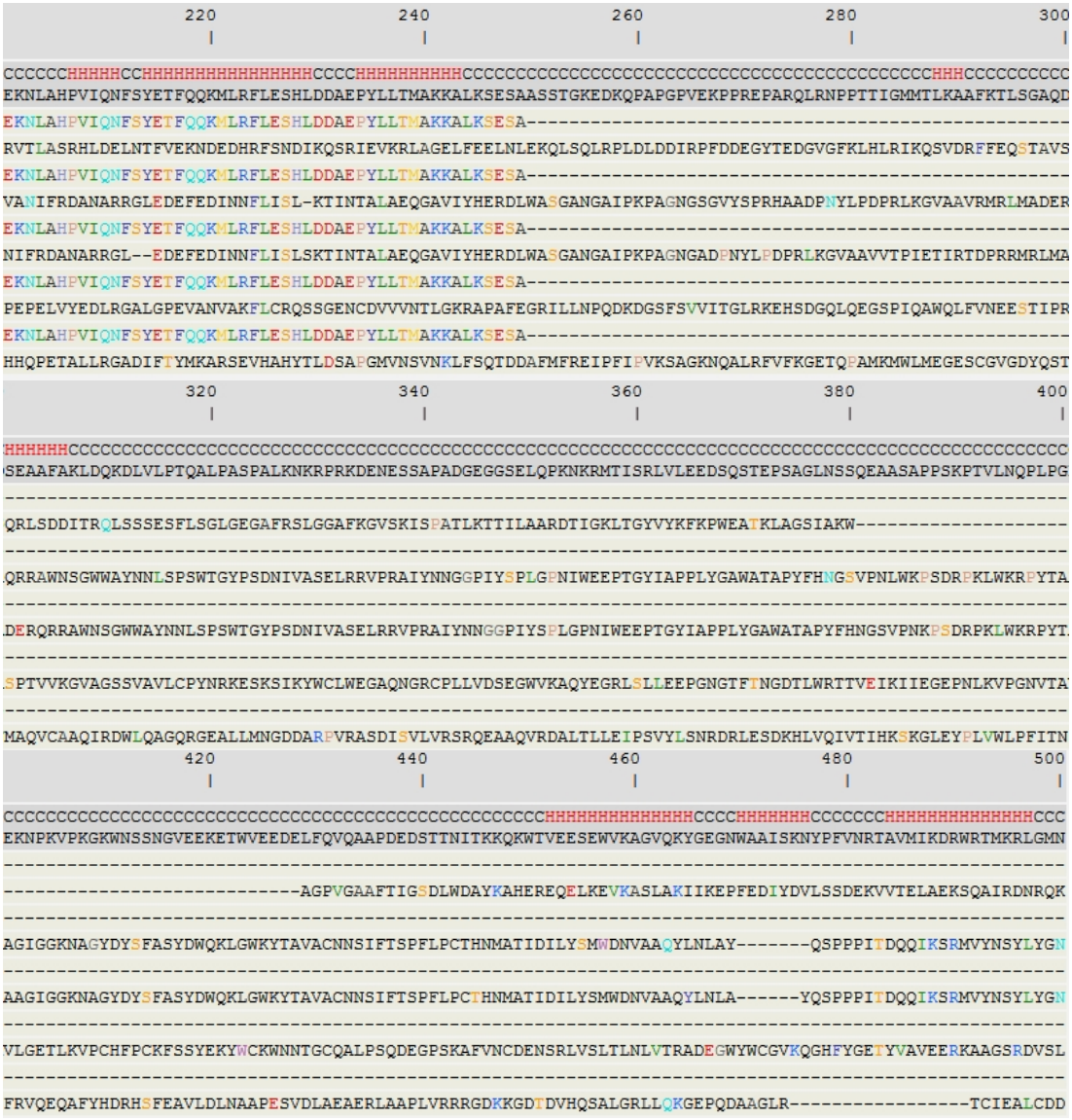


**Fig. 2. Multiple sequence alignment of ten best template sequences with hTRF2 protein**

The Stereochemical quality of that model was also verified with the tool MolProbity, VERIFY3D [45] and PROCHECK [46]. The Molprobity [47] score (>1) of hTRF2 was predicted 4.2 and Ramachandra favored residue was 80.56%. The second approach to access protein structures was to predict the 3D-1D score through VERIFY3D, which analyzes the compatibility of an atomic model (3D) with its own aminoacid sequence (1D). Each residue is assigned a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar, etc). A collection of good structures is used as a reference to obtain a score for each of the 20 amino acids in this structural class. The scores of a sliding 21-residue window (from -10 to +10) are added and plotted for individual residues. hTRF2

protein shows 0.43 for 3D-1D average score, the max value was 0.57 for the residue 34 and lowest was -0.16 for 89, 117, 118,119, 120. The modeled 3D protein structure was again verified through energy minimization steps (steepest descent and conjugant gradient) using GROMACS software. The steps helped to find the stable protein structure. After finishing the simulation run the energy values in terms of potential (-1.4842e+06 kcal\mol), kinetic (2.4312e+06 kcal\mol) and total energy (-1.0530e+06 kcal\mol) was calculated. The RMSD plot of backbone (Fig. 3.) was plotted to verify the stability of hTRF2. The plot shows that the initial fluctuation was conducted during the simulation up to time slot of 800-900ps but after that there was not such hike in the RMSD. After 1000ps fluctuation of RMSD was in the range of 1.3nm – 1.5nm. The RMSD curve also implies that the modeled structure was good amount of stable in water condition.

Different properties of hTRF2 was tabulated by CLC protein workbench and it shows percentage of positive residues is much higher (0.158) than negative residues. The aliphatic index (AI) of hTRF2 is 70.12, regarded as a positive factor for the increase of thermo stability of globular proteins. The computed pI value for hTRF2 (pI>7) indicated their basic nature. Kyte-Doolittle and Eisenberg scale was used to predict membrane spanning regions, antigenic sites, exposed loops or buried residues. But hTRF2 plot does not show any significant result in the above two scales. CYS_REC predicted the position of cysteines and their probable bonding pattern in the position 106, 118 and 165 residues with zero probability of disulphide bonds.

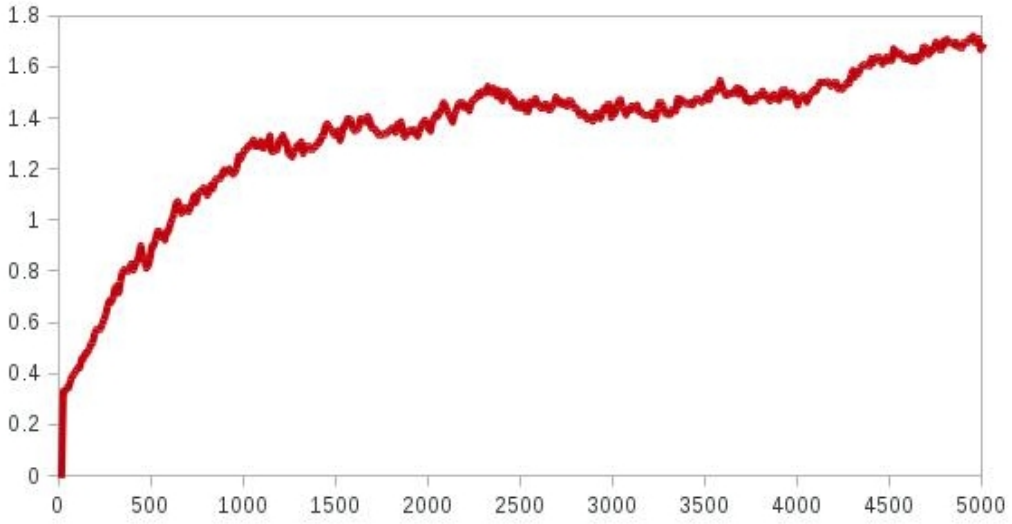



**Fig. 3. RMSD plot of hTRF2 protein in water with reference to 5000ps of time scale.**

The pre-requisite of DNA-protein docking study was the generation of double stranded 3D-DNA structure which was predicted by 3D-DART server. While binding of protein to DNA, large conformation changes exhibit [48] which can greatly alter the shape of the interaction surface. Knowing to this fact, the docking studies were carried out with different bend angles to find out the best docked orientation of hTRF2 and template DNA. Varying bend angle was selected 0º to 60º for defining the DNA-protein interaction more preciously. An animation of DNA strand (TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG) of hTRF2 protein is shown in the below figure (Fig. 4.).

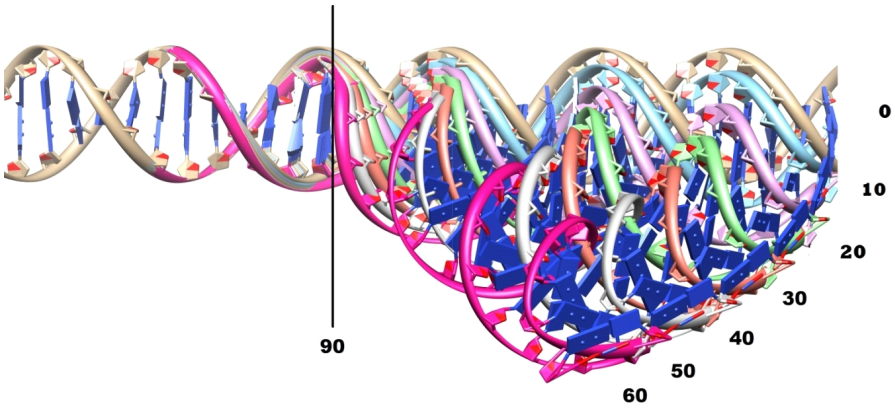



**Fig. 4. Stereo view of different 3D-DNA strand with varying bend angles (0º- 60º) and with tilt of 5º between 10 to15 nucleotides. The figure was generated in Chimera 1.6.2**

The result of interaction site was further applied for docking studies using HADDOCK server. Docking of DNA and hTRF2 started with the three selected regions (Table 3.) for protein and above designed DNA strands. With the entire above selected region docking studies were carried out with different DNA bend angle (0° to 60°). For each and every bend angle the active and passive residues were same for DNA. The best result was selected for docking studies was helix3 region (Fig. 5.) with DNA bend angle of 60°.

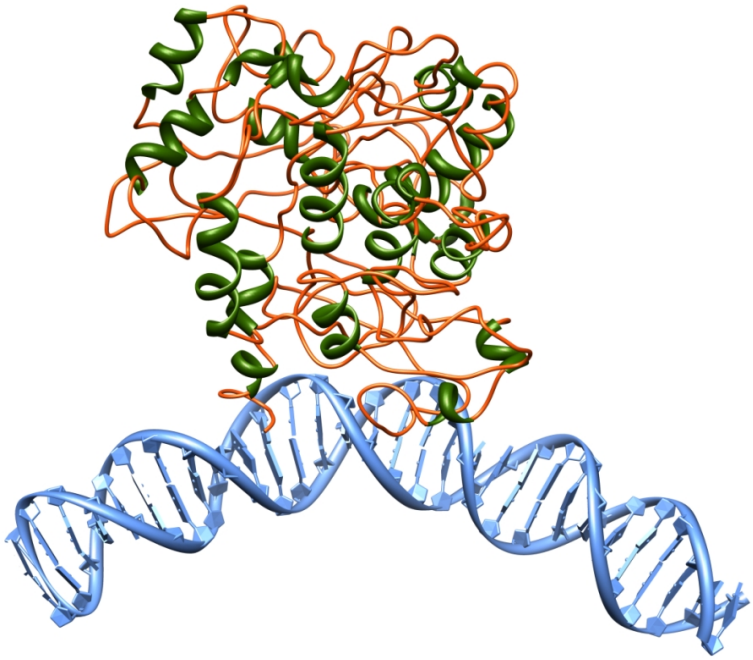


**Fig. 5. Complex structure of hTRF2 and DNA after docking**

The docking result was verified with minimum RMSD values and maximum Z-score. The docking result was verified with minimum RMSD values and maximum Z-score (Table 3.).

This docking result shows RMSD value (0.8 +/- 0.5) for 8 structures of 1 cluster with lowest restrain value of 180.2 +/- 69.05 and overall Z-score was -2.2. The violation analysis was the summation of calculated violation energy for distance restrain and H-bonds restrains. The other parameters (van darwaal, electrostatic and desolvation energy) in table 3 also indicate the overall bonded and non-bonded interactions of the docked structure. The following table also indicates the RMSD from the overall lowest-energy structure, which is comparatively less (0.8 +/- 0.5) from the other bend angles. The close up view of the interaction has also been shown (Fig. 6). It was reported that similar structure of DBD of hTRF1 and hTRF2 and the common residues are involved in making DNA protein interaction [17]. With the resemblance of this study, the following complex structure of DNA-hTRF2 (Fig. 5.) indicates some similar type of interactions. The residues like Arg and Lys are the basic amino acids and have the potential to make bonds with phosphate backbone of DNA. Arg490, Arg492, Arg496, Lys495 are coming in direct contact with Gua18, Thy 19, Thy20, Ade21 in DNA-hTRF2 complex. It can be recommended from the result (Table 3.) that during the bend angle 60º the proximity of H bond formation between positively charged amino acids and the phosphate backbone increase for DNA-hTRF2 complex with respect to other bend angles. It can also be suggested that with very specific binding some non specific binding can also be formed with the bases of other strand to stabilize the structures. The mutation in the binding residues can also alter the binding capacity of the receptor molecule [49,50]. In future the mutation can implement in the specific binding residues in hTRF2 DNA binding domain which can enhance the binding capacity of the overall DNA-protein complex.

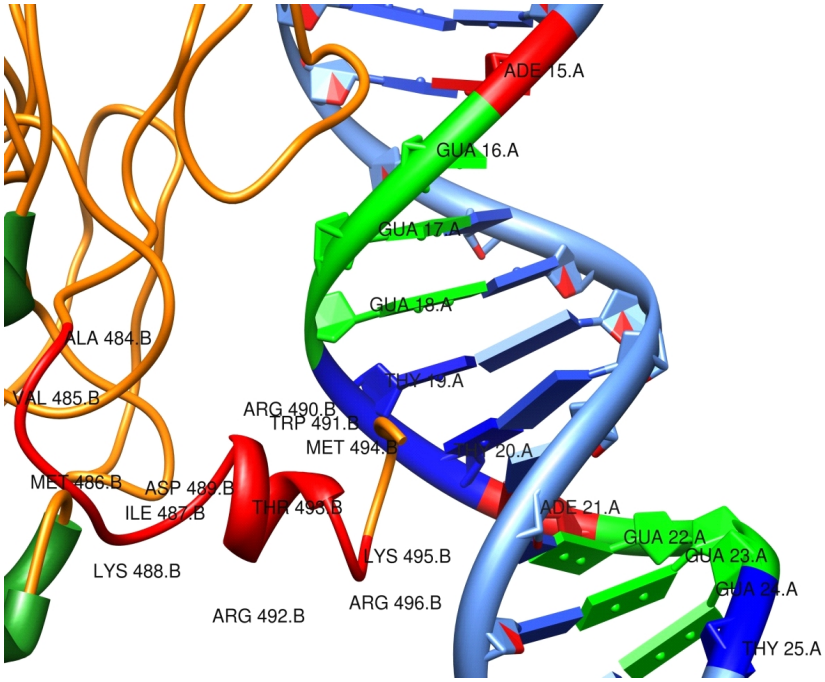



**Fig. 6. Close up view of the interaction was shown, where the interacting amino acids were highlighted with red color. Name and position of the amino acids and nucleotides were also represented here.**

**Table 3. Haddock result of hTRF2 and DNA template with different bend angles. The best docking result (60° bend) is shown here as bold letters.**

| Parameters | DNA bend angle 0° | DNA bend angle 20° | DNA bend angle 30° | DNA bend angle 40° | DNA bend angle 50° | DNA bend angle 60° |
|---|---|---|---|---|---|---|
| Haddock Score | -40.4 +/- 11.5 | -24.7 +/- 8.7 | -33.2 +/- 36.8 | -23.5 +/- 4.9 | -32.0 +/- 5.8 | -38.1 +/- 10.6 |
| Cluster Size | 8 | 11 | 5 | 9 | 26 | 8 |
| RMSD from the overall lowest-energy structure | 2.0 +/- 0.2 | 30.8 +/- 0.5 | 2.3 +/- 2.4 | 11.3 +/- 0.2 | 23.0 +/- 0.1 | 0.8 +/- 0.5 |
| Van der waals energy | -59.5 +/- 7.8 | -47.9 +/- 2.7 | -36.5 +/- 6.1 | -50.9 +/- 5.7 | -60.2 +/- 6.3 | -57.4 +/- 8.3 |
| Electrostatic energy | -301.1 +/- 36.0 | -277.4 +/- 49.2 | -538.6 +/- 109.6 | -284.7 +/- 39.5 | -255.2 +/- 16.0 | -236.9 +/- 9.5 |
| Desolvation energy | 59.8 +/- 4.4 | 64.4 +/- 9.2 | 92.6 +/- 11.7 | 57.9 +/- 10.3 | 59.9 +/- 5.3 | 48.6 +/- 10.8 |
| Restraints violation energy | 195.2 +/- 38.66 | 142.6 +/- 29.02 | 184.4 +/- 58.82 | 264.8 +/- 84.77 | 192.7 +/- 21.73 | 180.2 +/- 69.05 |
| Buried Surface Area | 1750.0 +/- 137.4 | 1450.7 +/- 82.4 | 1506.8 +/- 338.5 | 1735.0 +/- 127.7 | 1691.2 +/- 145.1 | 1651.6 +/- 154.2 |
| Z-Score | -1.1 | -1.5 | -1.9 | -1.2 | -1.7 | -2.2 |

## 4. CONCLUSION

hTRF2, a *Myb-type* protein is very essential for maintaining the chromosomal end. It shows similar structure and function with hTRF1 protein in vivo which is well known for protecting telomeric DNA. The full protein structure was modeled to make an insight of the interaction of full protein with modeled DNA structure (varying bend angle). The structure modeled by I-TASSER was also validated with well known MD simulation software package, GROMACS by a 5ns simulation run. From the result of Aliphatic Index (AI) (~71%) and hydrophobicity (~50%) it is cleared that hTRF2 is a compact globular protein. The active site of hTRF2 3D structure was classified in three parts to get a better docking result and shows the interaction with DNA major groove. For accurate result in DNA-protein docking the DNA was modeled with different bend angles and four active regions of protein were also selected. The best docked orientation result came out for the residues 485-499 with 60° bend angle. This docking result shows RMSD value (0.8 +/- 0.5) for 8 structures of 1 cluster with lowest restrain value of 180.2 +/- 69.05 and overall Z-score was -2.2. So this result suggests that during binding of hTRF2 with DNA, 60° bend angle is very much important issue and maybe this result verifies the *in vitro* interaction. From this *in-silico* study in future we can make changes in selected amino acids and further check the interaction with DNA. The study also helps other researcher to get a glimpse on hTRF2 protein full structure and its characteristics.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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