

Structural Modeling and Molecular Dynamics Simulation Studies of Camel Milk Kappa Casein Protein

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ABSTRACT

Kappa casein protein belong to phosphoprotein's casein family which contribute 80% of milk protein comprise of α S1, α S2, β -casein and κ -casein. Kappa casein gene (CNS3) is highly conserved in mammalian species and show variation containing 1997 base pairs. Kappa-casein involved in vital physiological processes. Along with the stabilizing coagulum by interfering with lactation cycle, it has many antimicrobial activities against *Escherichia*, *Helicobacter pylori*, *Listeria*, *Salmonella* and *Staphylococcus*, yeast and filamentous fungi. To date the X-ray structure of Kappa casein is not available. The sequence under accession no of **P79139** was retrieved from uniprot and 3D structure was built with template ATP-dependent RNA helicase p47 (PDB ID 1XT1.A) showing 43.4% sequence identity using MOE2010.11. The alignment of target and template protein was done using MUSCLE server. The structure was refined by subjecting to MOE-DYNAMIC tool and final structure was used for protein-protein interaction with *Helicobacter pylori* (data not shown).The consistency of modeled structure was confirmed by getting acceptable scoring from ERRAT and Rampage owing to high accuracy of MOE for modeling and dynamics simulation. Toward the inhibitory activity this modeled structure may unclothe the mechanism of eradication of helicobacter pylori from gastric epithelial cell.

Keywords: Homology modeling, Md simulation, Casein and Inhibitor

INTRODUCTION

Kappa casein (κ -casein) is a protein which is post transcriptionally modified by addition of one or more phosphate molecules. It belong to phosphoproteins's casein family divided into four groups α S1, α S2, β -casein and κ -casein [1] that contribute 80% of milk protein fraction. Among which the most abundant proteins are α s1-casein and β -casein at 10-12 mg/ml and 10 mg/ml respectively while α s2-casein and κ -casein are present at 3.7mg/ml and 3.4 mg/ml respectively [2]. Casein is secreted by mammary gland cells, genes of which are resides in chromosome 6 arranged in following orders: α -s1 casein, β -casein, α -s2 casein and κ -casein [3]. Kappa casein gene (CNS3) is highly conserved in mammalian species and show

variation containing 1997 base pairs. Kappa-casein involved in vital physiological processes. The efficiency of digestion upsurge by fragmented kappa casein into insoluble peptide (para kappa-casein) and a soluble hydrophilic glycopeptide in the gastro intestinal tract (GIT) prevents infant hypersensitivity to proteins and the activity of several pathogens was inhibited [4]. For example, human milk kappa casein inhibits *Helicobacter pylori* adhesion to sections of human gastric mucosa [5]. Stabilizing the coagulum was powered by Kappa-casein and it also has major Influences on milk composition and processing properties by associating with lactation cycle [6]. κ -casein peptide has been found to have antimicrobial activity against wide range of pathogenic organisms

such as *Escherichia*, *Helicobacter pylori*, *Listeria*, *Salmonella* and *Staphylococcus*, yeast and filamentous fungi [7]. Secondary structures have been found to be of great importance in antimicrobial effect [8].

In the absence of experimentally three dimensional X-ray structure, a homology model could provide a rational opportunity to obtain three dimension (3D) model [9, 10]. The application of homology model such as mechanism investigation and structure based molecular mechanism has been recognized. This approach gives a good model with template sharing more than 25% identity. The template used in this study was the X-ray structure of ATP-dependent RNA helicase p47 (PDB ID 1XTI.A) showing 43.4% sequence identity from homo sapiens. This template has two domains. The N-terminal domain consists of seven β -sheets surrounded by eight α -helix and C-terminal domain contains seven β -sheets surrounded by five α -helix. Our model showed similarity with N-terminal domain. The present study deals with the prediction of first 3-D model of kappa casein (*Camelus dromedarius*) based on comparative homology modeling using MOE 2010.11

MATERIALS AND METHODS

Retrieval of sequence

The primary sequence of Kappa casein (accession number: P79139) of *Camelus dromedarius* was retrieved in FASTA format from the Universal Protein Resource (UniProt) (<http://www.uniprot.org/>) and was saved in text file with name Kappa casein in local directory. The retrieved sequence of Kappa casein was described *in silico* using ExPasy-ProtParam tool [11].

Secondary structure prediction

Secondary structure of our protein under observation was predicted using different methods. The protein sequence was submitted to the following server and was analyzed for secondary structure. Servers used in this task were double prediction method (DPM) [12], Discrimination of protein secondary structure class (DSC) [13], PHD[14], Predator [15], SIMPA96 [16], SOPM [17], Self-optimized prediction method with alignment (SOPMA) [18], Sec. Cons [19].

Template selection

Similarity search was performed using PDB Search Tools suited in MOE 2010-11 against PDB databank of MOE. The sequence was pasted in Sequence Query and run the Search keeping parameters like Gap start -12, Gap Extend -2, E-value cutoff 10, E-value Accep 0.5, Z iterations 100 and Z cutoff 6 using identity Matrix. X-ray crystallographic structure of Probable ATP-dependent RNA helicase p47 (PDB ID 1XTI.A) was chosen as template sharing highest sequence identity of 43.4% with the target protein and was loaded to MOE Sequence Editor. This template consists of two domains. Our sequence showed similarity with N-terminal domain.

Target-template alignment

Sequence alignment of target protein kappa casein and template protein (PDB ID 1XTI.A) was performed by using MUSCLE [20] server (<http://www.ebi.ac.uk/Tools/msa/muscle/>). In parameters set the Output format to ClustalW.

Prediction of intrinsic disorder

In order to identify segments of disordered and higher flexibility, the tools DisEMBL[21], Globplot [22] and Regional order neural network (RONN) [23] were used. Protein disordered prediction is important in determining protein function and protein folding pathway [24, 25].

Homology modeling

The model was generated using MOE Homology tool. Chain 1 is our target sequence and chain 2 is template sequence. In model refining tool Intermediate was set to Medium, Final model to Medium, using scoring function Generalized Born/Volume Integral (GB/VI). The Force field was set to Amber99 with Solvation R-Field. Total of 10 models was generated, and the final model was loaded to MOE main window. The sequences were aligned before starting homology modeling by using MOE-Align application.

Dynamic simulation

The model generated by MOE Homology tool was subjected to MOE Dynamic tool in order to refine it. The energy of protein was minimized by using MOE Force field AMBER99 by concerning calculation toward solvation energy with Born implicit solvation. The energy was minimized to RMS Gradient 0.05. The overall charge of protein was optimized with partial charge option in MOE 2010-11. The other parameter was set to default values that were ensemble at NVT and algorithm at NPA for creating ensemble trajectory. The acceleration, velocity and position were saved after each 0.5 picoseconds (ps). Before starting MD simulation, addition of water molecule by Water Soak option with soak mode BOX and layer width 5 was followed by energy minimization. The system was heated from 0K to 300K in 20 picoseconds (heat time) followed by production time of 200 picoseconds. The system was cooled back to 0K in 20 picoseconds.

Validation of modeled structure

The stereo chemical quality of the generated model was initially evaluated by MOE package using Rampage. Then it was subjected to ERRAT [26] server to check the overall quality.

RESULTS AND DISCUSSION

The sequence of camel milk kappa casein was obtained from Universal Protein Resource (UniProt) under the accession no of P79139 and the obtained sequence was submitted to ExPasy-ProtParam server to study its physicochemical properties (<http://web.expasy.org/protparam/>). The protein has 182 amino acids with molecular weight of 20417.5 and estimated theoretical isoelectric point (pI) of 8.55. The Extension coefficient

of protein is 10555 indicates that at a definite wavelength how much light was absorbs by a protein [27]. It is used by spectrophotometer in purification of protein. The instability index (II) is 44.72 that displaying protein stability [28]. Instability index smaller than 40 for a protein is expected as stable. Protein may be unstable with Instability index more than 40. The hydrophilic nature is predicted by The Grand average of hydropathy (GRAVY) index value of -0.150[29]. The Aliphatic index is 90.49 indicating the relative volume of aliphatic side chains (leucine, valine,

isoleucine, and alanine). The thermostability of globular proteins is calculated by Aliphatic index [30].

Secondary structure prediction

Prediction of secondary structure ensures whether amino acids lie in strand, helix or coil. The sequence was submitted to different servers and results were obtained as shown in Table1. The inspection of the table show that random coil dominate over extended strand followed by alpha helix.

Table 1. Secondary structure prediction calculated by different tools.

Secondary Structure	DPM	DSC	Sec.Cons	SOPMA	PHD	Predator	SIMPA96	SOPMA
Alpha helix	10.99%	11.54%	10.99%	16.48%	1.65%	18.68%	15.85%	17.03%
3 ₁₀ helix	0%	0%	0%	0%	0%	0%	0%	0%
Pi helix	0%	0%	0%	0%	0%	0%	0%	0%
Beta bridge	0%	0%	0%	0%	0%	0%	0%	0%
Extended strand	35.16%	16.48%	17.03%	15.93%	25.27%	15.38%	13.66%	18.68%
Beta turn	1.10%	0%	0%	1.10%	0%	0%	0%	4.95%
Bend region	0%	0%	0%	0%	0%	0%	0%	0%
Random coil	52.75%	71.98%	68.68%	66.48%	73.08%	65.93%	69.95%	59.34%
Ambiguous states	0%	0%	3.30%	0%	0%	0%	0%	0%
Other states	0%	0%	0%	0%	0%	0%	0.55%	0%

Prediction of intrinsic disorder

Protein disorder can be defined as the region without regular secondary structure and of high degree of flexibility in protein chain [31]. Recently it has been assured that functional segments of polypeptide are mostly present outside of globular domain specially the region of intrinsic disordered [31]. Globular domains are regions of high ordered and contain regular

secondary structure. The cellular and structural aspects of these regions are unknown but it has been thought about it that it become ordered when bound to another molecule [32, 33]. The sequence was submitted to different online server having free access and the result of different server is given in Table 2 and was shown in Figure 1.

Table 2. Intrinsic disorders predicted by different server.

Server	Disordered	Disordered REM465	by Disordered loop/coil definition	by Disordered by HOTLOOP definition
GLOBPROT		4-42, 72-77, 201-205, 209-214, 223-228		
DISEMBL		166-182	15-29,40-84, 152,161-174	91- 16-25, 97-111, 116-132, 139-149, 160-182
RONN	21 - 33, 98 - 176			

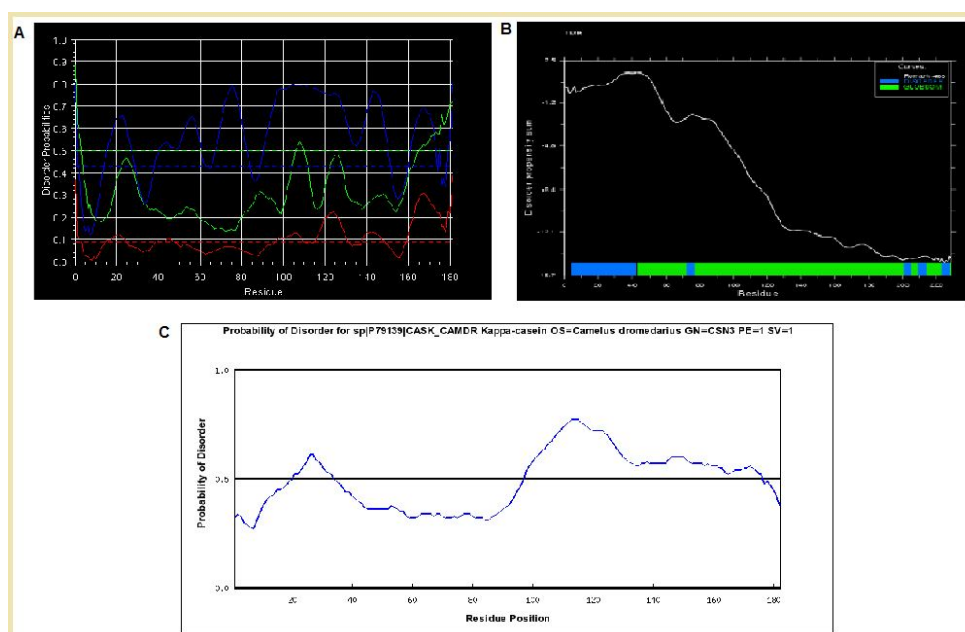


Figure 1. Protein intrinsic disorders predicted by different server (A) DisEMBL (B) Globprot (C) PRODOS.

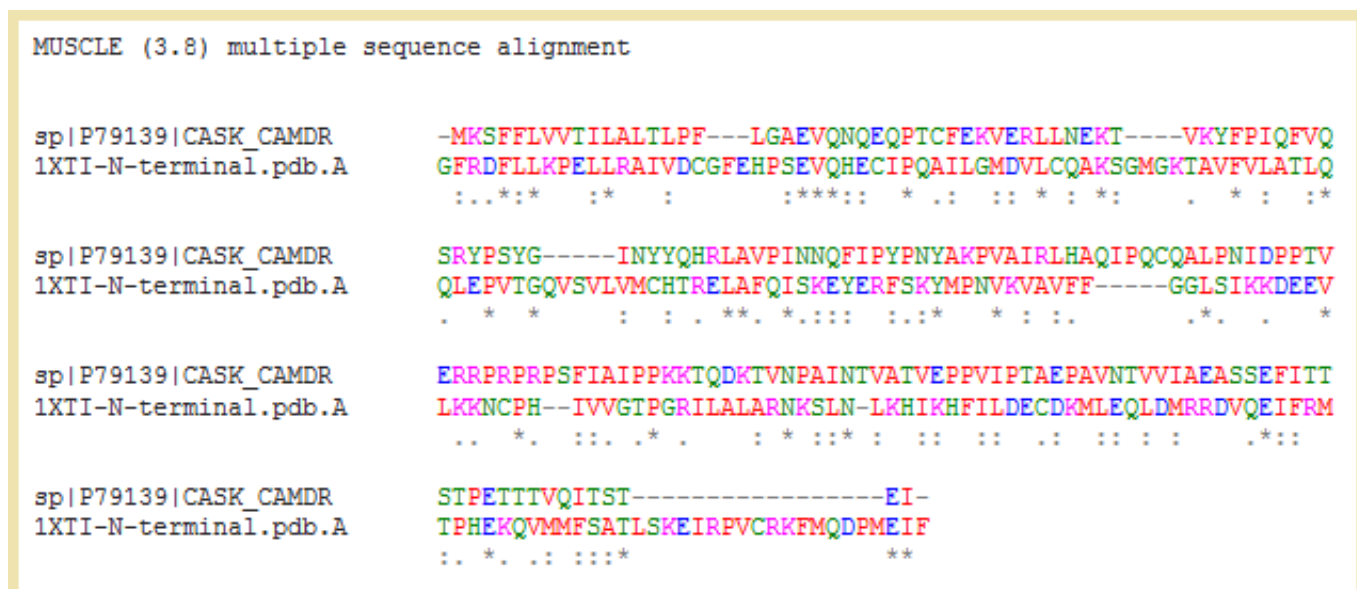


Figure 2. Sequence alignment between kappa casein and template 1XTIA N-Terminal.

Target-template alignment

The alignment of query sequence to template sequence was done by using MUSCLE [20] server. The alignment was done in order to generation a consequent model. The alignment was shown in Figure 2.

Homology modeling

To model 3D structure Molecular operating environment software (MOE 2010-11) was used which implement comparative protein structure modeling based on the following facts.

- 1) Initial partial geometry of target sequence was copied by MOE from template structure where residues identity was conserved.
- 2) By using a specialized logic insertions and deletions are treated for those residues having no assigned backbone coordinates [34].

- 3) Loops are modeled first in random order. A list of possible candidates were analyzed by using contact energy function chosen based on Boltzmann weighted averaging [35, 36]. Model was generated by keeping parameters like Model Scoring to Generalized Born/Volume Integral (GB/VI) methodology [37], Force fields to Amber99 supported in MOE recommended for protein homology applications [38]. After modeling, energy of structure was minimized to 0.05 Gradient by using Force field AMBER99 which is parameterized for proteins and nucleic acids and is not suitable for small molecules. The generated structure (Figure 3A) was saved in PDB format with suitable name. The generated model was superimposed on template structure with root mean square deviation (RMSD) of 1.242 angstroms showing a close homology (Figure 3B) using chimera version 1.7.

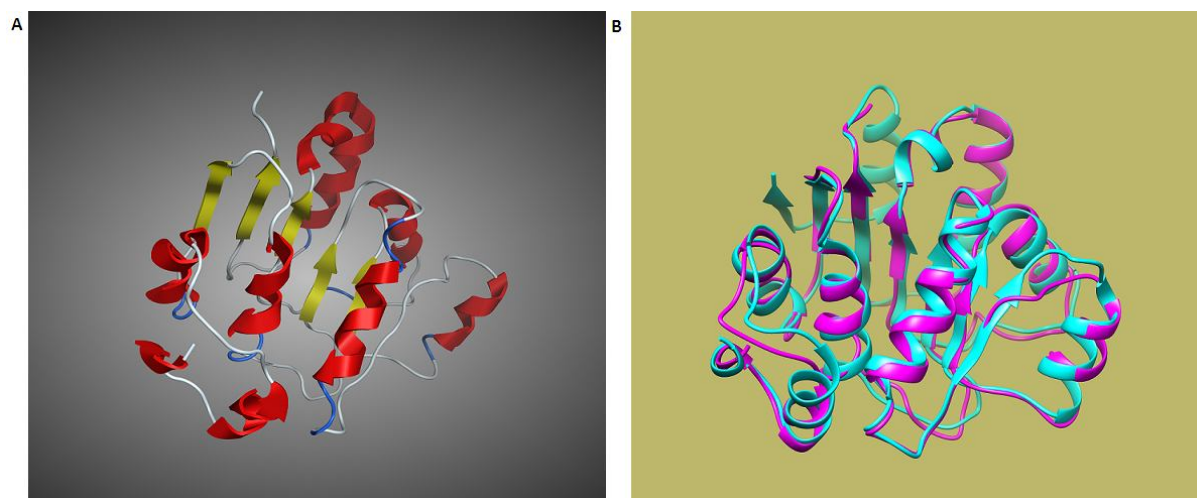


Figure 3. (A) Homology model for kappa casein (B) Superposition of target (magenta) and template (cyan).

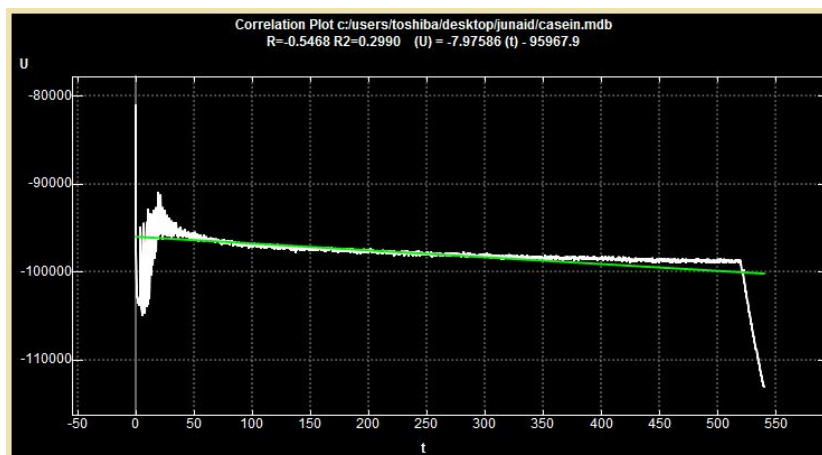


Figure 4. Potential energy plots during MD simulation at 300K.

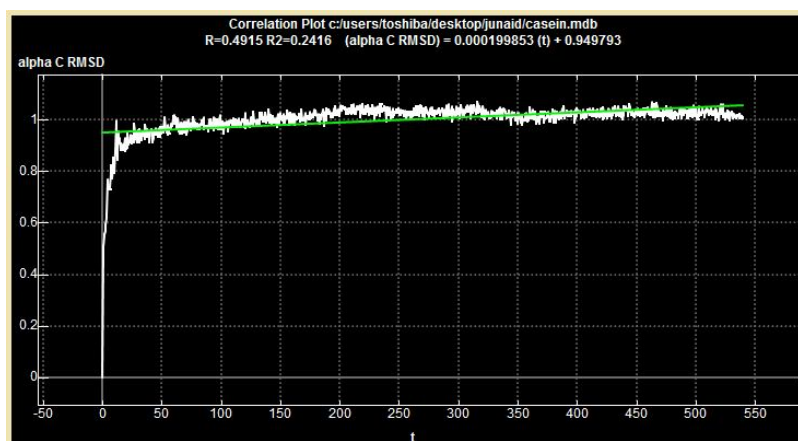


Figure 5. RMSD of alpha carbon versus time graph show equality after 350 picoseconds.

Dynamics simulation

Molecular dynamics simulation, which solve the classical equation of motion, results in conformational trajectories providing configurationally information to calculate thermodynamic properties of the system and explore the conformational space available to the system. The generated structure was further refined by subjecting to molecular dynamic simulation tool implemented into MOE-2010-11 [39]. The structure was saturated with partial charges followed by minimizing up to 0.05 RMS by using force field AMBER99 implemented in MOE-2010-11 [40, 41]. NVT ensemble was used as the temperature; volume and number of atom were held constant. The NPA algorithm was used as it is the most accurate and sensitive method in generating true ensemble trajectories [42, 43]. The Nose-Poincare-Anderson (NPA) method generate theoretically correct NVE, NVT, NPH and NPT ensembles (N, V, T, H, E, P) shows Number of atoms, volume, temperature, enthalpy, energy and pressure respectively). In heating phase the temperature was raised to 300K as is the normal human body temperature followed by production phase of 200 picoseconds in which temperature kept constant. After 150 picoseconds the system reaches the equilibrium indicating the validity of our protein at human body temperature. The system was then cooled for 20 picoseconds to get stable bonds energies. If it is not done, additionally energy minimization should be done.

There are many ways to analyses the MD simulation. In this study we were interested in potential energy plot of protein conformation with respect to time. Conformation mention the three dimensional arrangement of protein which can be change without fluctuating covalent bonds [44]. The potential energy plot verses time was given in Figure 4. From the analysis of trajectories as a result of MD simulation, After 350 picoseconds Root Mean Square Deviation (RMSD) of alpha carbon does not showed any fluctuation showing that system reached the equilibrium (Figure 5). The RMSD graph of backbone atoms as a function of time was also shown (Figure 6). The super position of initial and final structure after MD simulation was shown in Figure7 having 0.5 RMSD value, owing to greater stability of protein at 300 K and also showing the highly accurate modeling of MOE software.

Validation of modeled structure

The geometry of model was evaluated with Ramachandran plot calculation using Molecular operating environment (MOE 2010.11). Ramachandran plot revealed that 81.1% of residues are in favored region, 13.3% are in additionally allowed region and 5.6% are in outlier region (Figure 8C). The TYR63, GLN64, GLN92, ILE102 and THR139 were out of energetically favorable region. Ramachandran plot shows that total of 94% residues are in favored region

showing the high reliability of structure. The analyses of bond length of main chain backbone were also calculated in MOE 2010.11 revealing good quality of protein (Figure 8A). In Figure 8B the X-axis represents the sequential arrangement of amino acids and Y-axis shows the Z-Scores of the particular backbone bond length. Z score cutoff of 4 are drawn in red colour, above which the residue are in outlier region. Bond length calculation was presented in three separated plots. First plot is for the bond length of carbonyl carbon (pC) of previous amino acid with nitrogen (N)

and nitrogen with alpha carbon (C α) of next amino acid (pC-N and N-C α). Second plot is for bond length of alpha carbon with carbonyl carbon and side chain carbon (CB) with alpha carbon (C α -C and CB-C α). Third plot is for bond length of carbonyl oxygen and carbon (C=O) and carbon of side chain either alpha carbon. The errate score for our model is 92.529. Errat analyses the statistics of non-bonded interaction between different atoms and a score of normally 50 is acceptable. The score for our model showed the great accuracy (Figure 9).

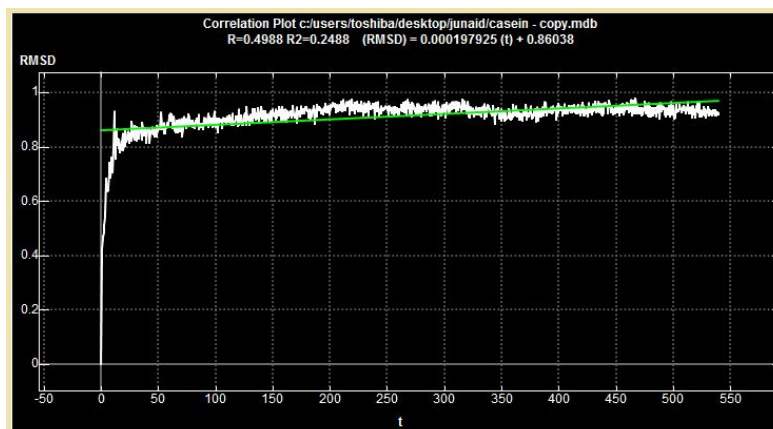


Figure 6. RMSD graph of backbone atoms versus Time graph show equality after 350 picoseconds.

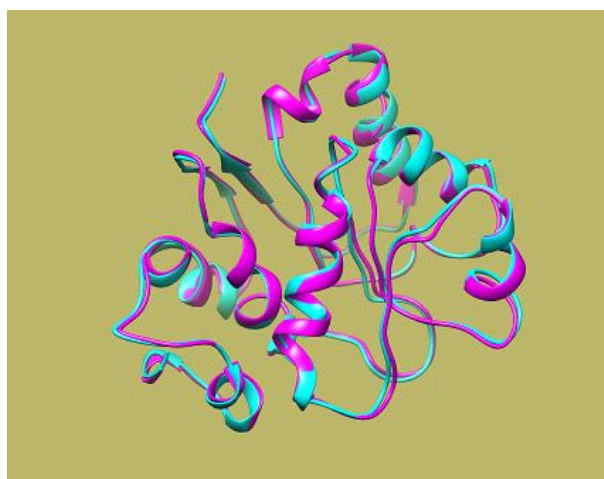


Figure 7. Superposition of initial and final structure.

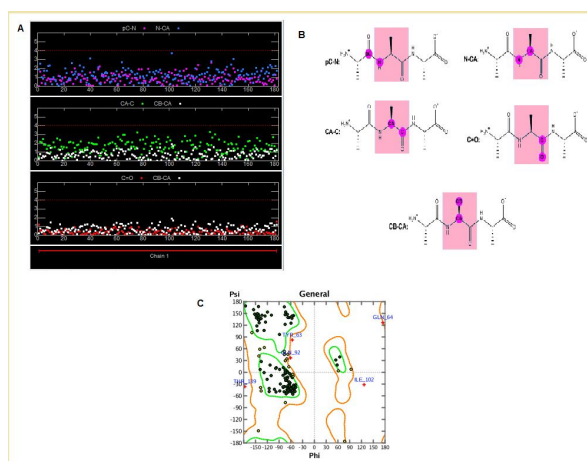


Figure 8. (A) Z-score of bond length (B) Bond length definition (C) Ramachandran plots.

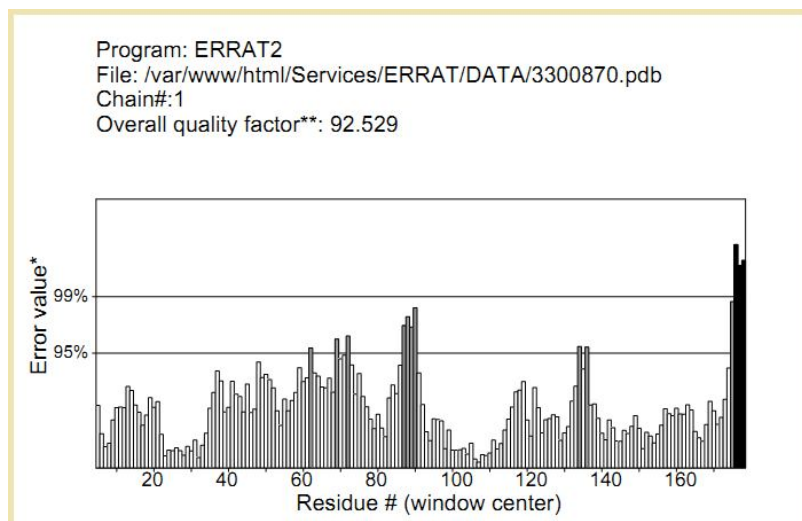


Figure 9. Overall Quality factor for kappa casein.

CONCLUSION

Protein structure enables the understanding of mechanism of protein's function. Solving the protein structure by experimental method is difficult process as it cannot meet the demand resulted from the exponential growth of protein sequences. Homology modeling is very important in drug design or in assessment of function to a protein as it gives insight into the interaction of protein with other proteins or with ligands. We modeled for the first time, the Kappa casein protein from camel milk which has a role in inhibition of *Helicobacter pylori* to gastric cell. MOE2010.11 was used for modeling purpose giving a good quality model. The quality of model was checked by Errat2 giving 92.529 quality factor, normally 50 quality factor is accepted. Furthermore, the model was refined by molecular dynamics simulation using MOE. The predicted three dimensional homology model might be used to uncover the mechanism of eradication of *Helicobacter pylori* from gastric epithelial cell.

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