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FLAP ENDONUCLEASE 1(FEN1) INTERACTION STUDY WITH PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) THROUGH HOMOLOGY MODEL AND DOCKING

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Abstract- PCNA and Fen1 proteins play a very crucial role within cell to complete replication process and other cell cycle activities. Absence of experimentally solved protein structures of PCNA and Fen1 of *Methanosarcina mazei* in archaeal domain of life inspire us to build homology models of these proteins to understand the interactions between these very important cell cycle proteins at the residue level. *M.mazei* is one of the rare archaeal organism that inhabit in diverse environmental conditions, having both prokaryotic and eukaryotic nature and also has the ability to produce methane. Interactions natures of these two core replication processing proteins in such a interesting organism were studied by docking of homology models, electrostatic surface potential of individual proteins and their complexes. Although evolutionarily human is more evolved organism but this study confirm PCNA and Fen1 interact similarly in *M.mazei* through a PCNA inter domain connecting loop and PIP-box motif of Fen1 protein. However, actual residues involved between these two proteins in two different domain of life have evolved and mutate over the course of evolution. So, from the present study we can conclude that the core replication processing proteins like PCNA and Fen1 interact in similar fashion whether it's a higher eukaryote like human or methane producing archaeal organism like *M. mazei* .

Keywords- PCNA, Fen1, Homology model, PCNA-Fen1 interaction, Methanosarcina mazei, Archaea

Introduction

Flap endonuclease 1 (Fen1) is an essential protein within a cell to maintain genomic stability of a species by participating in core replication process [1]. During replication process Proliferating cell nuclear antigen (PCNA) binds with Fen1 and increases the endonuclease activity of Fen1 up to 50 folds [2]. Within a cell, the interaction between Fen1 and PCNA during the replication process is believe to be important for PCNA to load Fen1 to the replication fork for sustainable Okazaki fragment maturation [3,4]. Fen1 is also involved in certain kinds of DNA repair, like "long patch" base excision repair, mismatch repair and some forms of recombination [2]. PCNA, which is also known as the 'DNA sliding clamp', substantially stimulated the activity of Fen1 in a in vitro experiments [5,6]. However, so far the structural basis of the Fen1-PCNA interaction is unknown in *M. mazei* as the experimentally verified structures of these core replication process proteins not yet available for this organism. Previous study showed mutation in Fen1 effects phenotypes in yeast with critical loss of certain functionality such as sensitivity to UV, loss of efficiency in chromosome segregation and accumulation in S phase of cell cycle. Mutations also interfere Fen1 from the proper interactions with PCNA, which reduces the cleavage efficiency of flap DNA at the replication fork [7], consequently producing a unfavorably long flap DNA strands [8]. Study showed PCNA has a remarkable feature to interact with multiple interacting proteins, which are also involved in neumerous metabolic processes of the cell like DNA synthesis, DNA repair, DNA methylation, DNA trnaslesion process, okazaki fragment maturation, cell cycle control and chromatin remodeling. Therefore, PCNA controls important metabolic pathways within the cell and to discover any changes in the nature of interactions is very important to properly reveal the complexity of that particular interactions.

The archaeon *Methanosarcina mazei* and related species are of great ecological importance as they are the only organisms which fermentate acetate, transforms methylamines and methanol to useful product like methane, carbon dioxide and ammonia (in case of methylamines) [9]. Only Methanosarcina species possess all three known pathways for methanogenesis and are capable of utilising a variety of methanogenic substrates, including acetate. They release methane into the global carbon cycle (methane is both a potential alternative energy source and a potent greenhouse gas). Since acetate is the precursor of 60% of the methane production on the planet, organisms like *M.mazei* contribute substantially to the formation of methane gas, e.g. in rice paddies [9]. Therefore, *Methanosarcina mazei* and other related organisms could be marked as villain as they are responsible in a limited way for global warming too. On the other hand utilizing the organism's ability to produce methane could be an organic solution as an alternative energy source for ever increasing energy needs in modern economy. Overall *M. mazei* is a very interesting and important organisms to study as a model organisms and already studied in several occasion previously [9]. In present study amazing archaeon *M. mazei* was chosen as a model organism to study the interaction nature of Fen1 and PCNA.

Experimentally verified protein structures is essential to study protein-protein interactions through computational method and as it is very difficult to determine the structures of protein complexes through crystal formation, therefore, often computational methods are widely used to predict protein-protein interactions [10,11]. Since no experimentally verified 3D protein structure of PCNA and Fen1 are available in *M. mazei*, homology modeling (or comparative protein structure modeling) techniques were used to build three dimensional models of a protein (target) from its amino acid sequence on

the principle of an alignment with a similar protein along with known structure (template) [12-14]. In this study homology modeling [15,16] technique was used to construct 3-D models of PCNA and Fen1 protein of *M. mazei* archaea.

It is important to develop computational docking methods that starting from the structures of template proteins can determine the structure of their complexes with accuracy close to that provided by experimentally determined structures. In the absence of experimentally verified structures of any one of the proteins of PCNA and Fen1, we have to obtain these structures through homology modeling and their interaction study through protein-protein docking method.

Thus investigating the nature of interactions of Fen1 protein with PCNA in methane producing archaea *M. mazei* will enable us to understand the complexity and similarity or difference of interactions with their eukaryal counterpart. The interactions of PCNA and Fen1 proteins are involved in core replication process and replication is essential for multiplying the cell and as a consequence, it indirectly controls production of various proteins and molecules. Thus, understanding structural features of PCNA-Fen1 complex in *M. mazei* will help one in bioengineering of this complex to regulate the emission of greenhouse gas as well as production of alternative energy through methane production.

Material and Methods

Target Sequence and Template

Methanosarcina mazei is our model organism for this study. PCNA and one of its interacting protein Fen1 were selected for the homology modeling and protein-protein docking study. Protein sequences of *M. mazei* for above mentioned two proteins were collected from NCBI (www.ncbi.nlm.nih.gov); the accession numbers are gi:23821929 (PCNA) and gi:28380015 (Fen1). Protein sequences of PCNA and Fen1 of M. mazei were used to Blast the Brookhaven Protein Data Bank (www.pdb.org). Any resulted proteins from the BLAST [17], which is structurally closer to the query proteins, based on sequence identity were included in template dataset. BLAST hit experimentally solved protein structures were selected as the templates, which show similarity percentage above 60 with query protein sequences and mostly when the organisms were also from archaeal domain of life. The Identity percentages were found as low as 38% for PCNA and 52% for Fen1. The other details of the different parameter of sequences and templates were listed in [Table-1].

Table 1- Template structure selected for building homology model and their properties.

M. mazei Protein	GI id	Template Crystal ID	Identity	Similarity	Length	Species
PCNA	gi:42543622	1RWZ	94/246 (38%)	152/246 (61%)	245	*Archaeoglobus fulgidus*
Fen 1	gi:6980604	1B43	178/340 (52%)	250/340 (73%)	340	*Pyrococcus furiosus*

(See Supplementary [Table-S1] and Supplementary [Table-S2]).

Table S1- Best hit crystal structure of PCNA proteins in *M. mazei*

Query Organism	GI id	GI id	Crystal ID	Identity	Similarity	Length	Species
Methanosarcina mazei	gi|23821929						
		gi:42543622	1RWZ	94/246 (38%)	152/246 (61%)	245	*Archaeoglobus fulgidus*
		gi:29726302	1IZ5	78/236 (33%)	138/236 (58%)	249	*Pyrococcus furiosus*
		gi|29726301	1IZ4	77/236 (32%)	137/236 (58%)	249	*Pyrococcus furiosus*
		gi|13096384	1GE8	77/236 (32%)	137/236 (58%)	249	*Pyrococcus furiosus*
		gi:50513815	1UD9	64/236 (27%)	125/236 (52%)	245	*Sulfolobus tokodaii*

Table S2- Best hit crystal structure of Fen1 proteins in *M. mazei*.

Query Organism	GI id	GI id	Crystal ID	Identity	Similarity	Length	Species
Methanosarcina mazei	28380015						
		gi|6980604	1B43	178/340 (52%)	250/340 (73%)	340	*Pyrococcus furiosus*
		gi|24987745	1MC8	179/340 (52%)	253/340 (74%)	336	*Pyrococcus horikoshii*
		gi|116668192	2IZO	165/340 (48%)	233/340 (68%)	346	*Sulfolobus solfataricus*
		gi|5821777	1A76	153/341 (44%)	217/341 (63%)	326	*Methanococcus Jannaschii*

1RWZ crystal structure was considered as template for building homology model of PCNA. Although low sequence identity (38%) was found with target *M. mazei* PCNA protein sequence, but it was the closest structure which was available in the databases. Moreover, 1RWZ also fulfilling all the basic criteria (like archaeal organisms and sequence similarity was 61%).

Target-Template Alignment

Alignment of query sequence with its template structure considered as the fundamental step towards homology modeling. The alignment of the query protein sequence with template crystal structure was constructed using the ClustalW [18] with the alignment of structure and sequence option on. This option was used for all the cases. These alignment files Supplementary [Fig-S1] and Supplementary [Fig-S2] were used for assigning protein properties to query sequences. Crystal structure were used only with higher identity percentage with the target protein sequence as a template for better assignment of protein fold and loop properties into the query protein sequences. Pair wise alignments of template and target sequence were used as input files in SWISS MODEL [19] web server (http://swissmodel.expasy.org) for alignment-mode options of homology modeling. In present study all target sequences exhibit alignment with their respective template structure.

Modeling and Energy Minimization

We have used SWISS MODEL web server to generate homology model of the target proteins. Energy minimization was performed for all the structures that were included in our studies, with the help of GROMACS 3.3.3 [20,21] molecular simulation and energy minimization package. The protein molecules of PCNA and Fen1 structures were embedded in a box of simple point charge water molecules with 1 nm separation between the box boundary and the solute. In our study energy minimization of solvent with the solute fixed was carried out with 500 steps of steepest descent followed by

2000 steps of conjugate gradient algorithm. Thus the structures obtained were subjected to 1 ns of molecular dynamics (MD) simulations in GROMACS at 300 K. For all the simulations steps GROMOS96 43a1 force field was used.

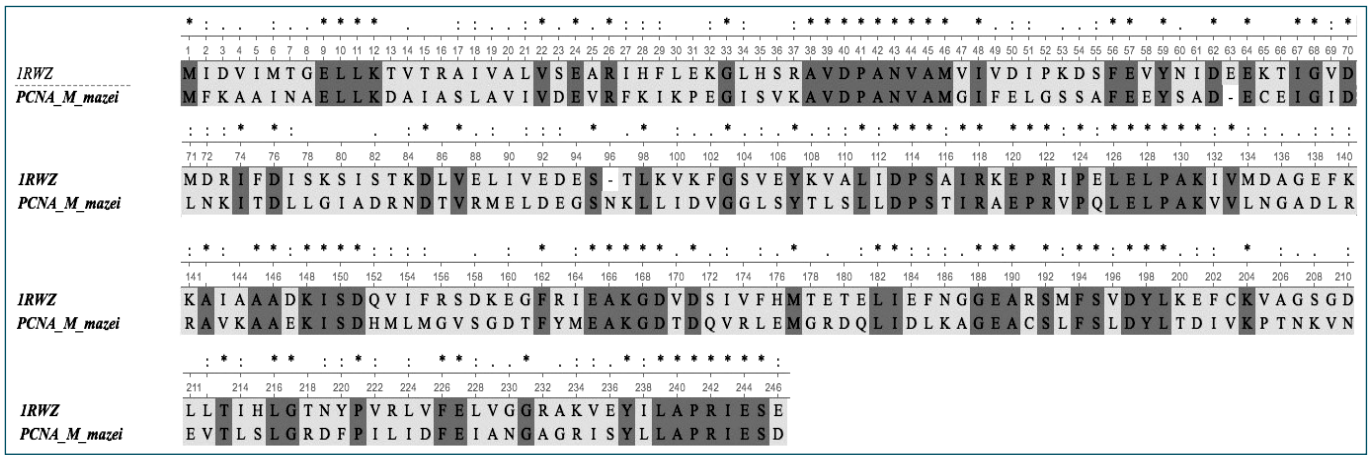

Fig. S1- Alignment of 1RWZ and *M. mazei* PCNA sequence

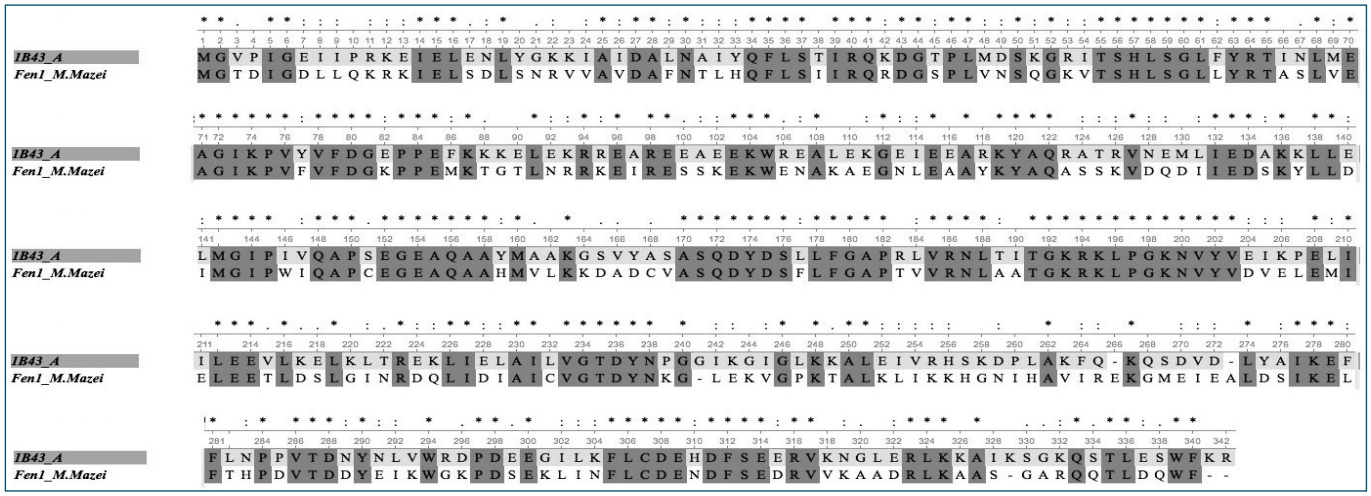

Fig. S2- Alignment of 1B43_A and *M. mazei* FEN1 sequence

Structure Validation

Homology model obtained from SWISS MODEL web server needs to be tested its steric correctness before including it as a candidate model. Therefore, we primarily filtered all of models with Verify3D [22]. The score of all homology models ranges between 0 and 1. Therefore, the scores indicate acceptable environment for the models. PROCHEK [23] was used thereafter to check steric correctness for all the models and one can include them as the candidate models if they fulfill the desired criteria such as more number of residues in the core region and no residues in the disallowed region. The best model was selected thereafter. To investigate how the model and the template structures were structurally similar and to check their overall structural deviations, the model and their template structures were superimposed with the server Superpose (http://wishart.biology.ualberta.ca/SuperPose/). Further, these models of PCNA and Fen1 were energy minimized followed by molecular dynamics simulation as described earlier.

Docking of Models

We have used homology models of PCNA and its partner Fen1 of *M. mazei* to build protein complexes through docking study. We used a well known web server ClusPro version 2.0 (http://cluspro.bu.edu) which incorporates docking program PIPER. PIPER is based on the FFT correlation approach. PIPER generally yields more near-native docked conformations than the other rigid body methods in ClusPro. The output of ClusPro 2.0 is PDB file containing the structures of ten models ranked as the most probable prediction candidate, according to the scoring function they have used. We considered lowest energy value when we choose the best probable complex structure among the results Supplementary [Table-S3]. All the complexes were then energy minimized as per the previous protocol mentioned in modeling and energy minimization section.

Table S3- Energy rank of complex between Fen1-PCNA in ClusPro server.

Cluster	Lowest Energy
6	-757.8
8	-734.4
1	-710.8
0	-692.6
2	-681.2
4	-676.8
7	-669.8
3	-663.1
9	-650.8
5	-641.6

Electrostatic Calculations

Adaptive Poisson-Boltzmann Solver (APBS) package [24] was used to calculate the surface electrostatic potential for each of PCNA and Fen1 by PyMol [25] software.

Results and Discussion

Primarily all models were filtered with Verify3D [22]. Verify 3D value usually ranges between -1 and 1, the profile score above zero in the Verify 3D graph correspond to acceptable environment of the model. Score near zero considered bad and the higher score near 1 indicate environment profile of the model was good. The Verify 3D score of all homology models in present study ranges between 0 and 1 and most part along the protein length were closer to 1. Therefore, the score indicate acceptable structural environment for the models.

Structural deviations of the model and the template structures were performed by CCP4 (http://www.ccp4.ac.uk/) program. Structural deviation was measured by rmsd calculation. The rmsd were measured between final positions of alpha carbons in the model and those of corresponding atoms in the templates. The rmsd value less than 1.5 Å were considered as an acceptable difference. In this study among all the models the maximum rmsd value was 0.49 Å, which was found in case of PCNA model when alpha carbon atoms were considered. The rmsd value for Fen1 was 0.240, which was again within the allowed range [Table-2]. Thus, the selected homology models for this study in comparison with the standard scale, considered to be good quality models.

Table 2- RMSD values of two model proteins.

Model Protein	RMSD C_alpha - C_alpha
PCNA	0.49
Fen1	0.24

The superimposed figures between model and template were given in [Fig-1] and [Fig-2]. With minute inspection18 β-sheets and 4 α-helixes were found in PCNA model. Within PCNA crystal structure 1RWZ, which was a *Archaeoglobus fulgidus* PCNA crystal structure, also consists of18 β-sheets and 4 α-helixes.

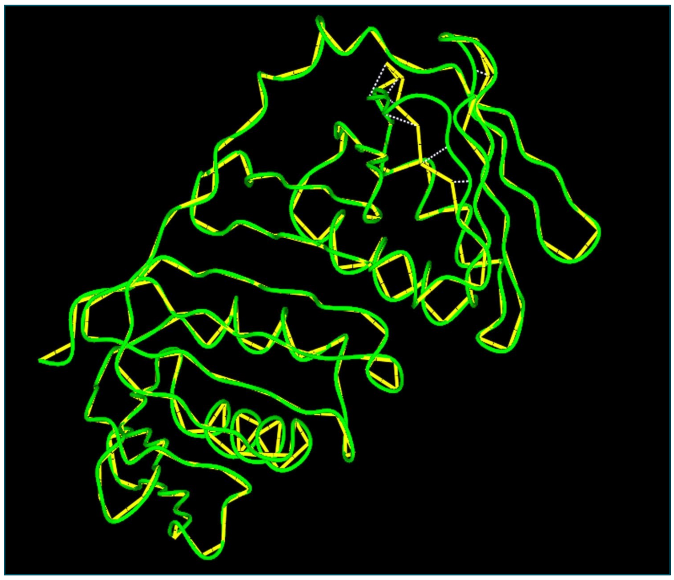

Fig. 1- Superimpose of PCNA model with template 1RWZ. Green colour representing template and yellow colour representing the model.

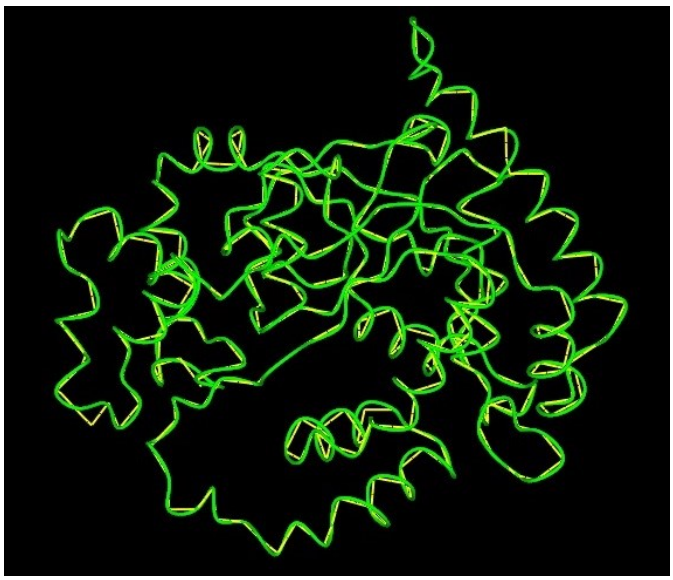

Fig. 2- Superimpose of Fen1 model with template 1B43 chain A. In above picture green colour for template and yellow colour for model.

Therefore, secondary structure of both the template and model PCNA was identical. Fen1 homology model of *M. mazei* consist of 9 β-sheets and 13 α-helixes. Crystal structure of Fen1 protein 1B43 chain A also consists of 9 β-sheets and 13 α-helixes. Therefore, secondary structures of selected models were in complete agreement with crystal structures [Table-1].

Comparative Docking Study

Models built by homology modeling were used for docking study as no experimentally verified structure of the complex was available in *M. mazei*. Model structures of PCNA and Fen1 proteins were docked to study the internal complexity involved in *M. mazei*. Many PCNA-binding proteins including Fen1, possess a short PCNA-binding motif, QXX(I/L/M)XXF(F/Y), this motif known as PIP-box motif is found either at the N or C-terminal end of the protein [6,26,27]. This long C-terminal tail represents one of the features common to eukaryotic Fen1. A comparative study was conducted between human PCNA-Fen1 (1U7B) complex with experimentally determined crystal structure and the docked complex of PCNA-Fen1 model from *M. mazei*.

Crystal structure of human PCNA-Fen1 complex, 1U7B revealed that C-terminal end of Fen1 came in close proximity with PCNA in the region of inter domain connecting loop (IDCL) of PCNA and also in loop region at residue 42-45 and in the C-terminal region of PCNA [Fig-3a] and [Fig-3b]. In C-terminal of Fen1, the conserved PIP-box motif was present spanning residue 331-338. Docking study of the PCNA-Fen1 complex of model structures in *M. mazei* revealed that C-terminal end of Fen1 also came in close proximity with PCNA in loop region [Fig-3c] and [Fig-3d] as found in human crystal structure. Here too, we observed the conserved PIP-box motif in the tail region and with a residue variant from human PIP-box motif. The seventh position of PIP-box motif of *M. mazei* harbors tryptophan (W) instead of phenylalanine (F), which we have found in PIP-box motif of human Fen1. It is interesting to note that tryptophan is less hydrophobic (-0.9) than the phenylalanine (+2.8) (Scale of Kyte-Doolittle [28]).

The residues at the interacting surface of PCNA-Fen1 in crystal

structure 1 U7B of human and docked complex of *M. mazei* are slightly different [Table-3]. In crystal structure 1U7B, the residue H44 and V45 of PCNA were in close proximity with the residue L340 of Fen1. Residue I128 from IDCL loop of PCNA came in close proximity with residue F44 of Fen1. Residues A252 and L254 in C-terminal end of PCNA came in close contact with the residues F343 and Q337, respectively of Fen1 protein structure.

However, in docked complex the residues A42, N43 and V44 of PCNA, from hydrophobic loop region, came closer with C-terminal end of PIP-box motif residues Q336 and L334 of Fen1 of *M. mazei*. Some residues [Table-4] from the IDCL loop region (residues 121-133), similar to crystal structure, also came in contact in docked complex of PCNA and Fen1 of *M. mazei*. Distances of contact residue were measured up to 10 Å as these contact residue distances remain similar in model and native structure of proteins [29].

Table 3- Contact residues of crystal structure 1U7B.

1U7B	Residue Pair in Complex with Distance				
Receptor (PCNA)	HIS 44	VAL 45	ILE 128	ALA 252	LYS 254
Distance	5.9 Å	5.6 Å	6.1 Å	7.7 Å	4.0 Å
Ligand (Fen1)	LEU 340	LEU 340	PHE 44	PHE 343	GLN 337

Contact residues were considered up to 10 Å.

Table 4- Contact residues of Model structure of PCNA-Fen1 complex.

PCNA-Fen1 Complex	Residue Pair in Complex with Distance				
Receptor (PCNA)	ALA 42	ASN 43	VAL 44	ALA 118	PRO 120
Distance	7.0 Å	5.9 Å	9.6 Å	6.3 Å	6.9 Å
Ligand (Fen1)	GLN 336	GLN 336	LEU 334	PHE 338	PHE 339
Receptor (PCNA)	ARG 121	PRO 123	LEU 125	LEU 125	GLU 126
Distance	9.9 Å	8.2 Å	4.8 Å	4.5 Å	3.3 Å
Ligand (Fen1)	LEU 334	GLN 331	PHE 338	LYS 324	GLY 328

Contact residues were considered up to 10 Å.

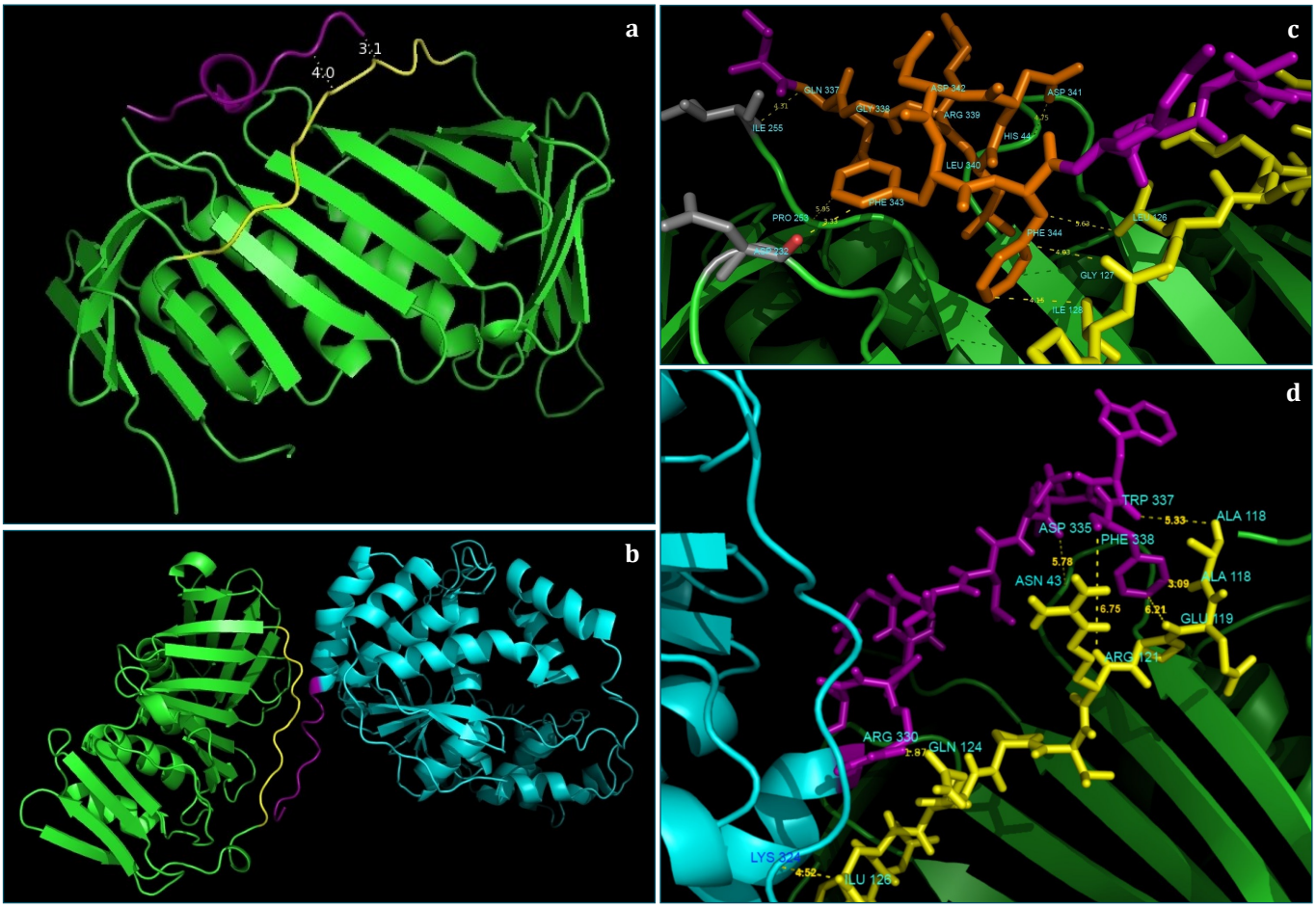

Fig 3- (a) and **(c)** Crystal structure 1U7B of PCNA (green) bind with Fen1 (purple) and contact region of PIP-box of Fen1 with IDCL (Inter domain connecting loop) of PCNA (yellow). **(b)** Docking of Homology model PCNA (green) and Fen1 (magenta) of *M. mazei* and **(d)** contact region of docked PCNA and Fen1 at PIP-box region (IDCL in yellow and PIP-box in purple).

The interacting residues of crystal structure of human and PCNA-Fen1 docked complex of *M. mazei* were found to be different. In crystal structure 1U7B (human) we have found three region of contact residues such as from hydrophobic loop region (residue 41-45), from IDCL loop region and from C-terminal end [Table-3]. However, in PCNA-Fen1 complex of *M. mazei,* residues from hydrophobic loop region, such as V44 was different comparing the crystal structure 1U7B, where it was H44. In the IDCL loop region it was I128 residue that was in contact region in crystal structure 1U7B, but in complex, residue I128 was not found to be involved in contact region, but, different residues like LEU125 and GLU126 of IDCL loop region were found to be involved in interaction [Table-3]. In C-terminal end of PCNA, residues like A252 and K254 were involved in contact region of crystal structure 1U7B. However, no such residues were found within the 10Å cut-off range of PCNA-Fen1 complex of *M. mazei*. Inspite of the differences in residues, mode of interaction between these two proteins in C-terminal end of Fen1 show similar nature of interactions pattern in crystal structure 1U7B of human and in docked complex of PCNA-Fen1 of *M. mazei*. Both in crystal structure and in model complex, the C-terminal end of

Fen1 possesses conserved PIP-box motif that interact with IDCL region and hydrophobic loop region of PCNA, almost in similar fashion. Although, evolutionarily human PCNA and *M. mazei* PCNA are far apart from each other, still each PCNA conserved their interactions as well as functions.

Electrostatic Surface Potential of Individual PCNA and FEN1 and Their Complexes

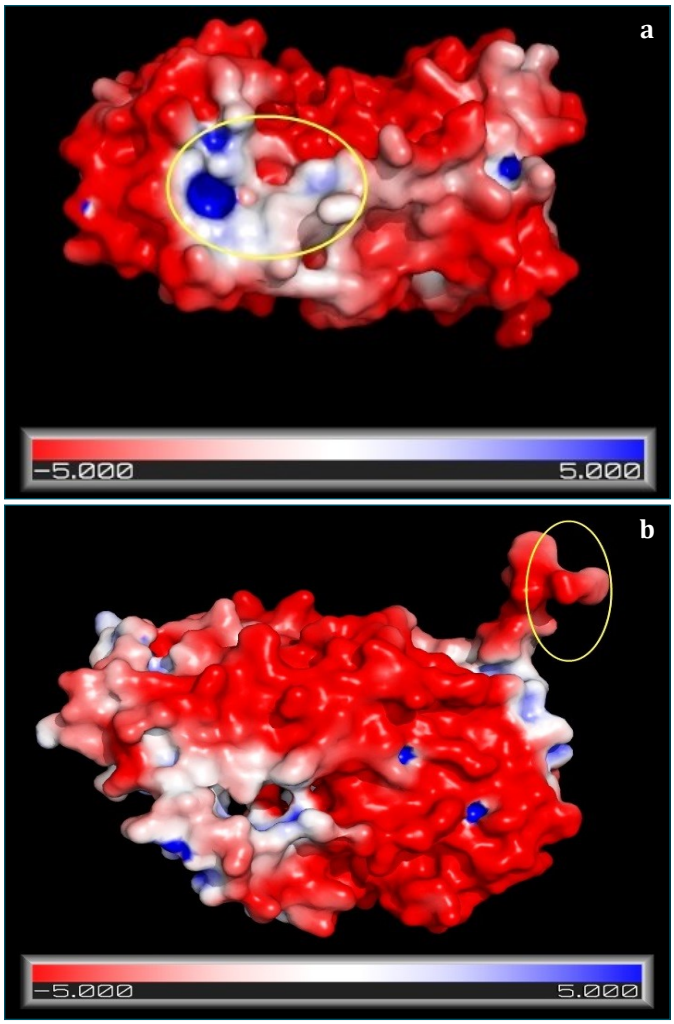

Fig. 4- Electrostatic surface potential of PCNA and its two interacting proteins. **(a)** Electrostatic surface potential of PCNA. **(b)** Electrostatic surface potential of Fen1. Electrostatic surface potential on solvent accessible surface around PCNA and Fen1 respectively. The surface colors are clamped at red (-5) or blue (+5). The positively charge blue region marked in [Fig-4a] and highly negative red region in [Fig-4b] signifies the area where they interact with PCNA (marked with yellow circle).

We calculate the electrostatic surface potential of our models to test the complementary nature of interacting surfaces between these proteins. It is evidently clear that the electrostatic surface potential of interaction site for PCNA [Fig-4a] and [Fig-4b] at the hydrophobic pocket region show highly positive to neutral while C-terminal end of Fen1(marked with yellow circle in both cases) show highly negative, coloured in red to slightly positive, coloured in blue. Therefore, electrostatic surface potential of interacting PCNA and Fen1 were complementary in nature. The colour scheme details of surface electrostatic potential and scale were given in [Fig-4b].

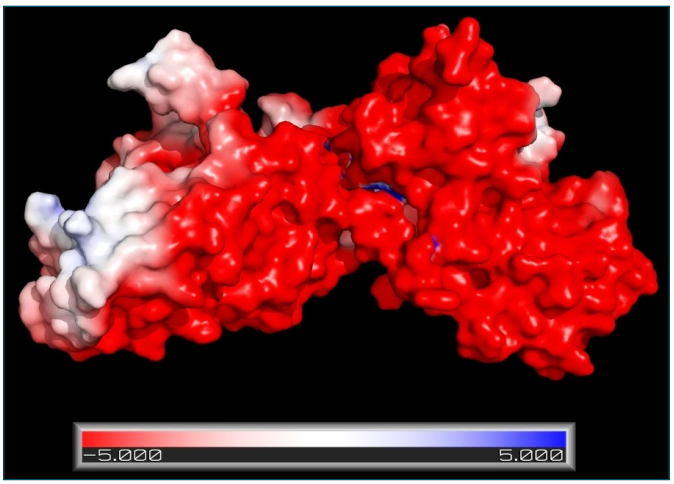

Fig. 5- Electrostatic surface potential of complex PCNA and Fen1. In the complex of PCNA-Fen1, the hydrophobic region of Fen1 (yellow circle [Fig-4b] the protein on the left side docked with the hydrophobic pocket of PCNA (yellow circle [Fig-4a]) on the right side protein in the [Fig-5]. Interacting surface of PCNA and Fen1 proteins show complementary electrostatic potential.

Conclusion

In this work we built homology models of PCNA and Fen1 of *M. mazei* successfully. We also docked the two proteins to study their interactions and identified the specific interacting residues of PCNA-Fen1 complex in *M. mazei*. It is found that the nature of PCNA-Fen1 interactions of archaeal *M. mazei* is grossly similar to eukaryal organism, human. In both cases - known and modeled structures, they interact through IDCL region, a hydrophobic loop region at residues 41-46 and with C-terminal end. Therefore, the basic structure of PCNA and its interaction with Fen1 remains more or less conserved from archaea to higher eukarya like human. Fen1 proteins also have the conserved PIP-box motif, the key interaction motif with PCNA from *M. mazei* to human. However, the interacting residues in human and *M. mazei* are different. The functions of proteins are determined by their structures and interactions among themselves. Since *M. mazei* produce methane which is an organic solution as an alternative energy source therefore it would be interesting to alter some of key interaction residues from both these proteins and regulate the replication process (since PCNA is a core replication process protein) and indirectly control greenhouse gas and production of alternative fuel.

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