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Modeling and Molecular Docking Studies on Rnase *Aspergillus niger* and *Leishmania donovani* Actin: Antileishmanial Activity

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ABSTRACT

A.niger Rnase was designed from ACTBIND (PDB ID: 3D3Z). Yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG) was used as template for *L. donovani* actin protein for 3D model in Modeller9v8. These models were testified by PROCHECK, ERRAT, WHAT-IF, PROSA2003 and VERIFY-3D. All evidences suggest that the geometric quality of the backbone conformation, energy profile, residue interaction and contact of the structures were well within the limits of reliable structures. The interaction energy of docking was calculated using the HEX server. Etotal and calculated RMSD values were -1.902, -9.323 kcal moL^{-1} and 0.402 Å, respectively. The study presented here has an advantage to design molecules that may have antileishmanial activity.

Keywords: *A. niger* Rnase, *Leishmania Donovani* Actin, Protein-Protein Docking, Modeller9v8, Hex Server, Novel Target For Leishmanisis

1. INTRODUCTION

Trypanosomatids cause various lethal forms of tropical human diseases including Leishmaniasis, which is caused by over twenty different species of *Leishmania* parasite (e.g., *Leishmania donovoni*, *Leishmania infantum*, *Leishmania major*, *Leishmania mexicana*). There are mainly three forms of the disease namely, cutaneous, visceral and mucocutaneous, out of which cutaneous Leishmaniasis is the most common while visceral Leishmaniasis is the lethal form. Leishmaniasis causes several clinical disabilities like disseminated visceral infection (Kala azar), ulcerative skin lesions and destructive mucosal inflammation, which impose a great social burden (especially for women), impair economic productivity and impede social development. Vector of the disease is female Phlebotomine sandfly, a dipteran, which transmits the parasite to human during blood sucking (Myler and Fasel, 2008). *Leishmania* parasite exists mainly in two life cycle forms namely promastigote in sandfly and amastigote in human macrophage. According to current WHO statistics about 12 million people living in 88 countries, mainly of 5 continents i.e., Asia, Europe, Africa, South America and North America are suffering from Leishmaniasis with 1.5-2 million new cases annually (Desjeux, 1992). This disease is endemic in low-income population of Central and South American countries (Tempone *et al*., 2005). Commonly available drugs for Leishmaniasis have severe side effects, high cost and low efficacy

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(Shukla *et al*., 2010). Thus, there is an urgent need for new and less toxic treatments for Leishmaniasis.

Currently, the fields of biochemistry, molecular biology, genetics and pharmacology have grown considerably in their ability to identify specific biological targets. Computational tools have recently been used to explore such targets in designing new drugs with the aim to decreased illness. Docking and molecular dynamics are the most commonly used computational tools for elucidation of leishmaniasis targets. Using the above tools, it is easier to find out the interactions and dynamics of drug and target at molecular level (Peitsch *et al*., 2000).

Actin is a cytoskeletal protein present within all eukaryotic cell types. The cell cytoskeleton is known to provide the basic infrastructure for maintaining cell morphology and functions such as adhesion, motility, exocytosis, endocytosis and cell division. The actin cytoskeleton and its regulatory proteins are crucial for cell migration and movement in most cells. The mechanism of cell movement involves actin remodeling which actually produces necessary force for cell migration. However, it has been observed that the alteration of actin polymerization or actin remodeling plays a pivotal role in regulating the morphology and phenotypic events of cancerous cells as a result of activation of oncogenic signaling pathways e.g., Ras and Src (Pollard and Borisy, 2003; Rao and Li, 2004) Gundampati *et al*., 2011a). Thus, elucidation of the molecular mechanisms of actin reorganization is important target for cancer therapeutics.

Leishmania is a protozoan organism which belongs to the family of trypanosomatid parasites (Glew *et al*., 1998). It contains microtubules, rather than the actin network, as its major cytoskeletal component (Gull 1999). Although the presence of actin gene has long been reported in both the *Leishmania* and Trypanosoma (Ben Amar *et al*., 1988)

De Arruda and Matsudaira (1994), but the existence of actin has only recently been convincingly demonstrated in these organisms (Shi *et al*., 2000; Sahasrabuddhe *et al*., 2004). However, not much is known about the structure and function of the actin network in these parasites, except that actin is required in the endocytic pathway, especially vesicular traffic, in T. brucei (Gracia-Salcedo *et al*., 2004).

Ribonuclease displays a variety of biological functions such as degradation of RNA, control of gene expression, cell growth and differentiation, cell protection from pathogens and apoptosis. Besides, ribonuclease works as potential anti-tumor drugs due to their cytotoxicity and uniquely influences several functions in the tumor cells. It has been observed earlier that the various ribonucleases from different sources such as onconases (Rana pipiens), bovine seminal RNase (Bovine seminal fluid), RNase T1 (*Aspergillus oryzae*), α-sarcin (*Aspergillus giganteus*), RNase P (Cultured human cells), ACTBIND (*Aspergillus niger*) B1 (CMI CC 324626) and RNase T2 (Cultured human cells) have been used for the treatment of cancer (Roiz *et al*., 2006).

In the present study, the 3-D structure of *A.niger* ATCC 26550 RNase and *Leishmania donovani* actin have been constructed using the templates ACTBIND (PDB ID: 3D3Z) (Gonzalez and Almog, 2008) and structure of the yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG) (Schutt *et al*., 1993), respectively. The resulting FASTA sequence was used to build the 3-D structures. Further, the molecular models of *A.niger* ATCC 26550 RNase and *Leishmania donovani* actin was constructed using Modeller9v8 package for homology modeling. The model quality was assessed using PROCHECK, PROSA, ERRAT, VERIFY 3D and WHAT-IF. The overall scores were used to choose the final model. Protein-Protein docking was performed between the molecular models of *A. niger* RNase and *L. donovani* actin. The detailed analyses of probable inhibition as well as interaction of the models were performed with high binding affinity. The studies presented in this manuscript will be useful to design molecules that may have antileishmanial activity.

2. MATERIALS AND METHODS

All the calculations were performed on a workstation Hi-end server: Pentium IV 3.4 MHz, AMD Athlon 64 bits dual processor with 4GB RAM and video graphics card. Molecular modeling tasks were performed with Modeller9v8 (http://www.salilab.org/modeller/9v8); protein-protein docking calculations were performed with HEX. For homology modeling of the *A.niger* RNase and *L. donovani* actin, the crystal structures of Actinbind a T2 RNase (PDB ID: 3D3Z) and structure of the yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG) respectively were used as templates (Gonzalez and Almog, 2008; Schutt *et al*., 1993). If not otherwise stated, default settings were used during all calculations.

2.1. Sequence Alignments

To find out an appropriate template structure for constructing the target model, the sequence of *A.niger* RNase (Gundampati *et al*., 2011b) and *L. donovani* actin were obtained from National Centre for Biotechnology Information (NCBI). With the aim of finding an adequate template for homology modeling of *A.niger* RNase and *L. donovani* actin, sequence alignments of its amino acid sequenc e against Protein Data Bank (Berman *et al*., 2000) were performed by means of the

BLAST algorithm (Altschul *et al*., 1990; 1997). The BLASTp alignment between the selected templates *A.niger* RNase and *L. donovani* actin was further refined using sequence alignments in the ClustalW 2.0.12 with default parameters (Thompson *et al*., 1994).

2.2. Molecular Model Building

The search using the BLASTp alignment algorithm within the PDB database showed various potential templates for molecular modeling purposes. More than 70 crystallographic structures were found to show high identity score with respect to *A.niger* RNase and *L. donovani* actin. Among them, ACTBIND (PDB ID: 3D3Z) and Structure of the yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG) structures were selected as templates for *A.niger* ATCC 26550 RNase and *L. donovani* actin, respectively. The 3D structures of *A.niger* ATCC 26550 RNase and *L. donovani* actin were predicted by homology modeling on the basis of the structures of 3D3Z and 1YAG was using the program modeller 9v8. This program is an automated approach to comparative modeling by satisfaction of spatial restraints (Sali and Overington, 1994; Sali, 1995; Sali *et al*., 1995). The modeling procedure begins with an alignment of the sequence to be modeled (target) with relative known three-dimensional structures (templates). About 100 models were generated and among them the one having lowest Root Mean Square Deviation (RMSD) value when superposed onto the templates 3D3Z and 1YAG, was chosen for further analysis (Guex and Peitsch, 1997).

2.3. Validation of the Homology Model

After the construction of the model, its quality was assessed considering both geometric and energetic aspects using PROCHECK (Laskowski *et al*., 1993) ERRAT (Colovos and Yeates, 1993), WHAT-IF (Vriend, 1990), PROSA 2003 (Sippl, 1993) and VERIFY 3D (Bowie *et al*., 1991) for internal consistency and reliability. The ramachandran plot computed with PROCHECK provided the residue position in particular segment based on the dihedral angles. Finally, the best-quality models were subjected to further calculations and molecular modeling studies, binding site analysis and other calculations.

The Structurally Conserved Regions (SCRs) between reference proteins were then identified and superimposed and the *A.niger* RNase sequence was then aligned to the SCRs using the alignment module and finally fine-tuned manually. Any one of the two segments for each SCR could be chosen to assign coordinates to *A.niger* RNase and *L. donovani* actin models. Then the chosen models were subjected to energy minimization in order to obtain a stable, low-energy conformation.

2.4. Protein-protein Docking

A docking study was conducted to evaluate the predictive ability of the *A. niger* RNase homology model and its relevance for use in the structure-based drug design studies. In order to perform protein-protein docking between the models of *A.niger* RNase and *L. donovani* actin generated model PDB's were submitted separately to online server HEX at its default parameters. HEX is an interactive protein docking and molecular superposition program; it works on FFT correlation using spherical polar coordinates and Gaussian density representation of protein shape. The computational part of the server consists of a 32-node cluster running the CentOS 5.2 operating system and using the OAR batch scheduling system (http://oar.imag.fr/). Each node consists of two quad-cores Intel Xeon 2.5GHz CPUs and eight of the nodes are equipped with two Nvidia Tesla C1060 GPUs. Hence, a total of 256 CPU cores and 16 GPUs are currently available on HEX server (http://www.loria.fr/~ritchied/hex_server/). The parameters used for the docking process were Correlation type-Shape only, Calculation Device- GPU, Number of Solutions-100, FFT Mode-3D fast lite, Grid Dimension-0.6, Receptor range–180, Ligand Range–180, Twist range-360, Distance Range-40. The drug and its analogues were docked with the receptor using the above parameters. The software Pymol (http://pymol.source-forge.net/) and Visual Molecular Dynamics (VMD) are very flexible, extensible packages for molecular visualization used to generate clear, informative and attractive representation of atomic data (Humphrey *et al*., 1996). There have been many efforts to predict protein-protein interaction binding sites based on the analysis of the protein surface properties (Aytuna *et al*., 2005) analyzed the surface patches using six parameters: solvation potential, residue interface propensity, hydrophobicity, planarity, protrusion and solvation accessible surface area. The six parameters were then combined into a global score that gave the probability of a surface patch forming protein-protein interaction.

3. RESULTS

3.1. Sequence Alignments

The sequence alignment was performed using the ClustalW 2.0.12 for homology modeling as shown in **Fig. 1**. The figure reveals that the residues involved in binding of various feedback inhibitors in template 1YAG (Gly-13, Ser-14, Gly-15, Met-16, Lys-18, Arg-62, Gly-

74, Gly-156, Asp-157, Gly-158, Val-159, Gly-182, Arg-183, Thr-202, Thr-203, Ala-204, Arg-210, Lys-213, Glu-214, Gly-301, Gly-302, Met-305, Phe-306 and Lys-336) were conserved in *L. donovani* actin. Similarly, the conserved amino acid residues involved in the binding of *A.niger* RNase and corresponding catalytic residues of template 3D3Z (Ala-159, Gly-160 and Ile-161) (Gundampati *et al*., 2011a) have been identified.

3.2. Molecular Model Building

The appropriate template was chosen based on sequence similarity, residue completeness and crystal resolution. Hundred models were generated and the model showing the least RMSD with respect to trace (Cα atoms) of the crystal structure of the template was saved for further refinement and validation **Fig. 2**. Refinement was performed in order to obtain the best conformation of the developed model of *A.niger* RNase and *L. donovani* actin.

3.3. Model Quality Assessment

The first validation was carried out using Ramachandran plot analysis computed with PROCHECK by checking residue-by-residue stereochemical quality of the protein structures. The analysis showed that residues of *A. niger* RNase, 3D3Z, *L. donovani* actin and 1YAG in the most favorable region were 100, 86.5, 95.4 and 94.4% and in the additional allowed region were 0, 13, 4.6 and 5.6%, respectively (**Table 1 and Fig. 3a, 3b**). The Root Mean Squares Deviation (RMSD) between the Cα atom of the template 3D3Z and the model was 0.89Å and the template 1YAG and the model was 0.94Å, which indicated high structural homology. Thus, the selected structure of *A. niger* RNase and *L. donovani* actin were found to be reasonable and reliable conformation was used for further protein docking. The selected structures were depicted in **Fig. 2**. The total quality G-factor 0.3 indicated a good quality model (acceptable values of the G-factor in PROCHECK are between 0 and -0.5, with the best models displaying values close to zero). The PROCHECK stereochemical analysis showed no bad contacts and no bad scores for main-chain or side-chain parameters.

The detailed secondary structural investigation of the predicted *L. donovani* actin model with PDB sum, a secondary structure prediction server revealed 74 (19.7%) residues were in strands, 136 (36.2%) in α-helices, 22 (5.9%) in 3-10 helix and 144 (38.3%) in other conformations (**Fig. 4a**). The tertiary structure of *L. donovani* actin showed close resemblance to 1YAG with a backbone RMSD value 0.60 Å. It was found that using the integrated sequence alignment tools and structural superposition algorithms, a target sequence can be mapped onto the modeling templates in one step. Then the initial sequence alignment can be optimized manually while the anticipated changes in the model backbone were reflected in real-time in the displayed structural superposition. By applying structural superposition and RMSD evaluations, our model appeareds very similar to the experimental one. The superposition of the average structure of the *L. donovani* actin with the initial model did not showed major structure conformational changes in comparison to the initial model, which in turn was consistent with the relatively low RMSD values (**Fig. 4b**). The overall low RMSD values for backbone superposition reflected the high structural conservation of this complex through evolution, making it a good system for homology modeling.

ERRAT is also called "overall quality factor" for non-bonded atomic interactions and higher scores mean better quality (Colovos and Yeates, 1993). The normally accepted range for a high quality model is >50 (Laskowski *et al*., 2005). In the current case, the ERRAT score for the *L. donovani* actin and *A.niger* RNase models were 84.239 and 84.615, respectively and fit well within the range of a high quality model. Whereas the ERRAT score for the templates 1YAG and 3D3Z were 98.039 and 97.391, respectively **Table 1** Thus, the above analysis suggested that the backbone conformation and non-bonded interactions of *L. donovani* actin and *A.niger* RNase homology models were all reasonable within a normal range.

The final evaluation of the built *L. donovani* actin structure was checked by VERIFY 3D (Bowie *et al*., 1991). The VERIFY 3D analysis indicated a reasonably good sequence-to-structure agreement because none of the amino acids had a negative score (average score = 0.36). It should be noted that compatibility scores above zero correspond to acceptable side chain environment.

WHAT-IF is used to check the normality of the local environment of amino acids (http://www.loria.fr/~ritchied/hex_server/). For the WHAT-IF evaluation, the quality of the distribution of atom types was determined around amino fragments. For a reliable structure, the WHAT-IF packing scores should be above -5.0 (Vriend, 1990). In this case, none of the scores for each residue in the homology model was lower than -5.0 as depicted in **Table 2**. Therefore, the WHAT-IF evaluation also showed that the homology mode structure was very reasonable. Analysis of the energy minimized *L. donovani* actin model with WHAT-IF web interface revealed that RMSD Z-Scores for bond angles and bond lengths were all close to 1.0 and also within the limits of templates. The interaction energy per residue was also calculated by the PROSA, 2003. Program (Sippl, 1993). In this analysis, the interaction

energy of each residue with the remainder of a protein was computed to judge whether it fulfills certain energy criteria or not. The PROSA Z-Score indicates overall model quality. Global analysis of the model *L. donovani* actin with PROSA showed a Z-Score of -9.44, indicating no significant deviation from typical native structures of similar size as template when compared with Z-Scores of -9.96 for 1YAG template. **Figure 5a** displays the PROSA, 2003. energy profiles calculated for the *L. donovani* actin model along with the templates. The energy profile of the *L. donovani* actin homology model was consistent with a reliable conformation based on its similarity to that of the template 1YAG. Evaluation of the energy minimized model of *L. donovani* actin with PROSA-web revealed that the Z-score value was-9.44 (**Fig. 5b**) in the range of native conformations of the crystal structures. PROSA-web analysis had showed that overall the residue energy of the *L. donovani* actin model was largely negative except for some peaks in the middle region.

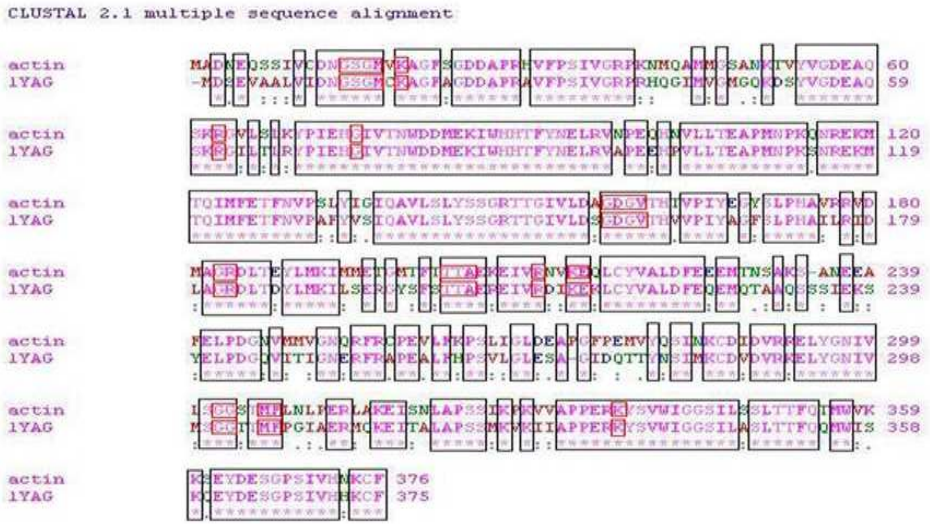


Fig. 1. Sequence alignment of *A.niger* RNase with the residues are crystallized 3D3Z. Highly conserved represented in rectangular boxes

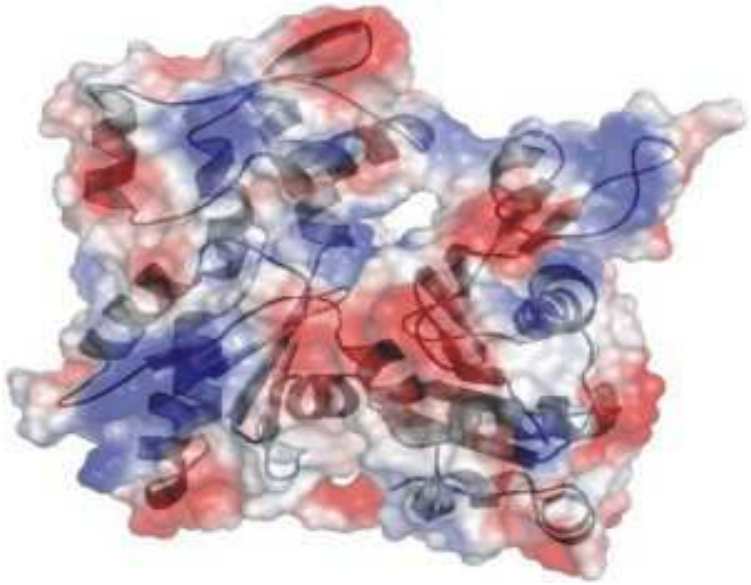


Fig. 2. 3D Structure of *L. donovani* actin in electrostatic representation

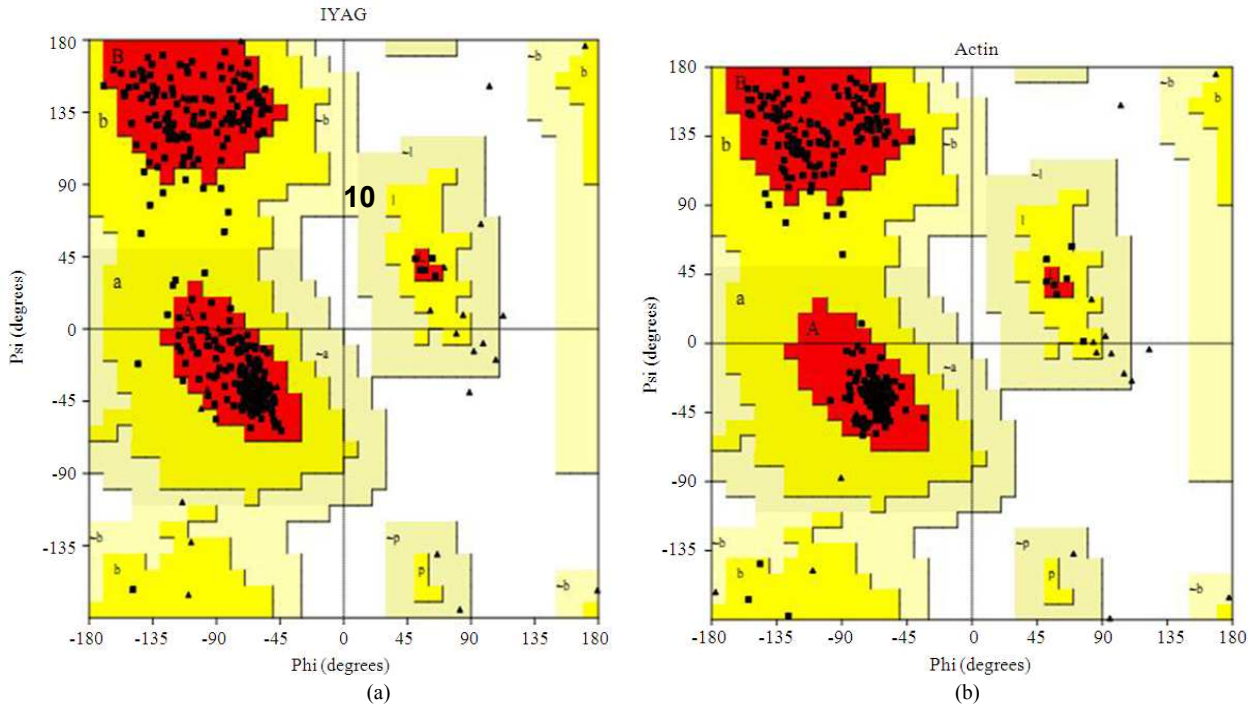
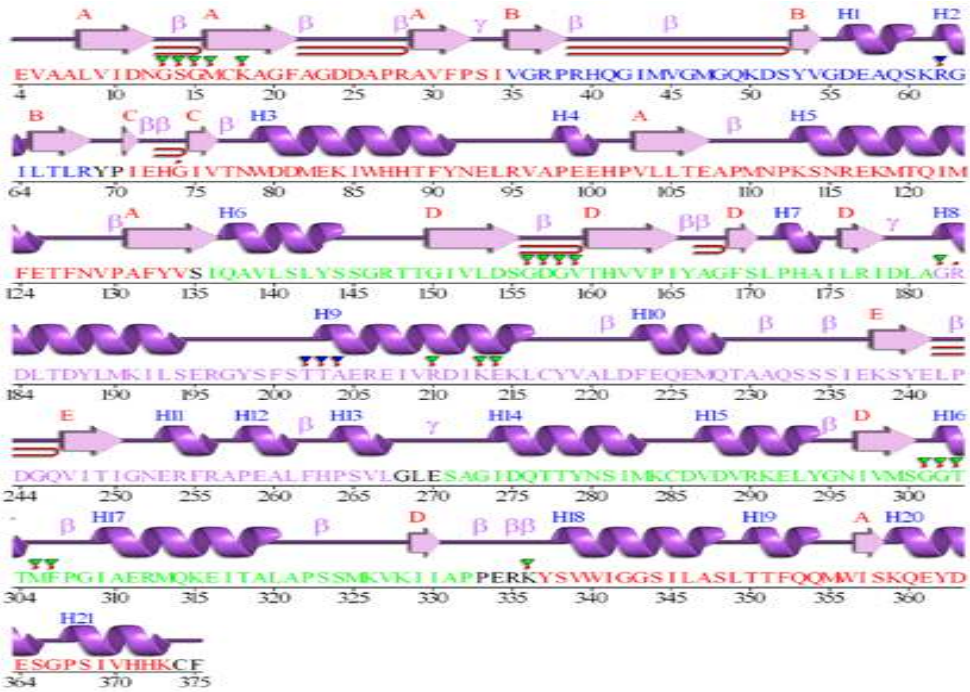
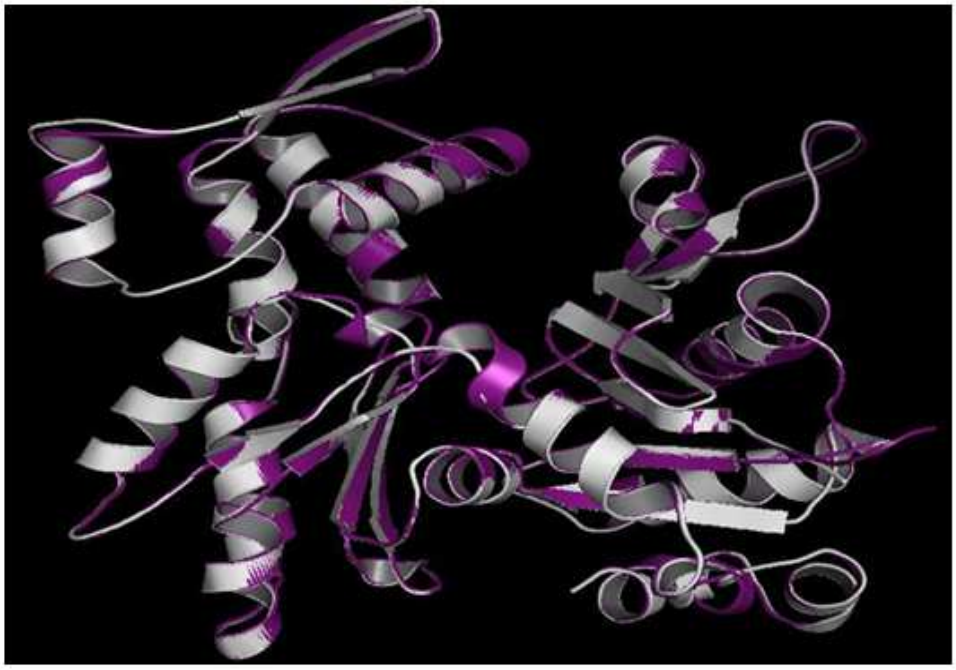


Fig. 3. (a) Ramachandran plot of template 1YAG. (b) Ramachandran plot of *L. donovani* actin, in which 311 reisudes in most favoured region (95.4%), 15 residues are additionally allowed regions (4.6%) and reaming residues generously allowed and disallowed egions (.0%)



(a)



(b)

Fig. 4. (a) Secondary structure of template 1YAG. (b) Secondary structure of developed model of *L. donovani* actin. c. Superposition of the average structure of *L. donovani* actin. The structures are presented as cartoon diagram. The average and the initial structure structures are colored green and magenta purple, respectively

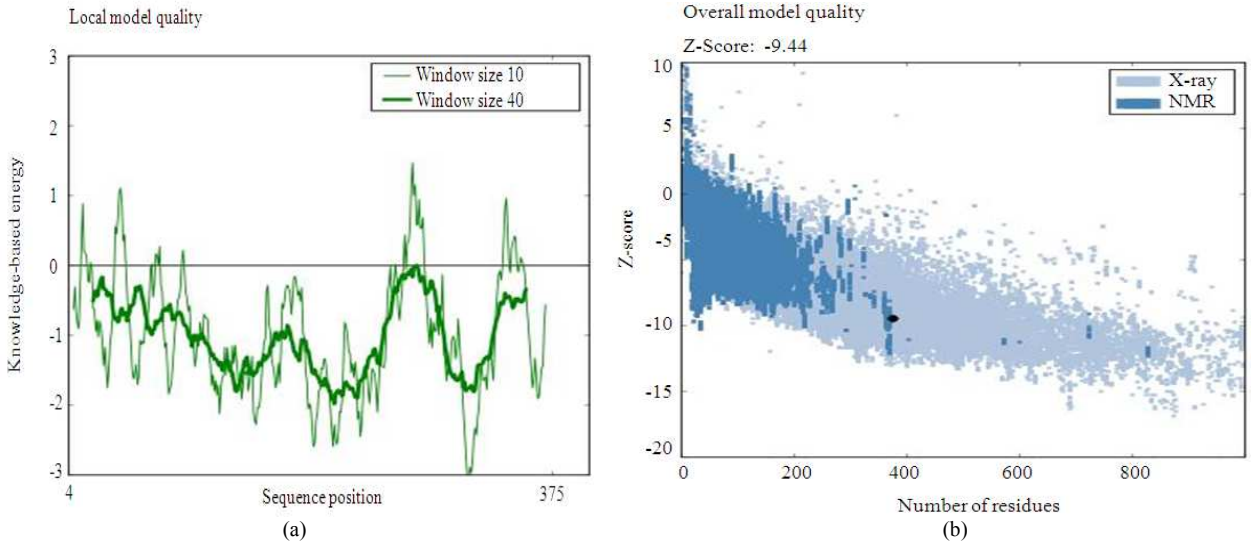


Fig. 5. (a) Energy plot for the predicted *L. donovani* actin. (b) PROSA-web Z-scores of all protein chains in PDB determined by X-ray crystallography (light blue) and NMR spectroscopy (dark blue) with respect to their length. The Z-score of *L. donovani* actin was present in the range represented in large black dot

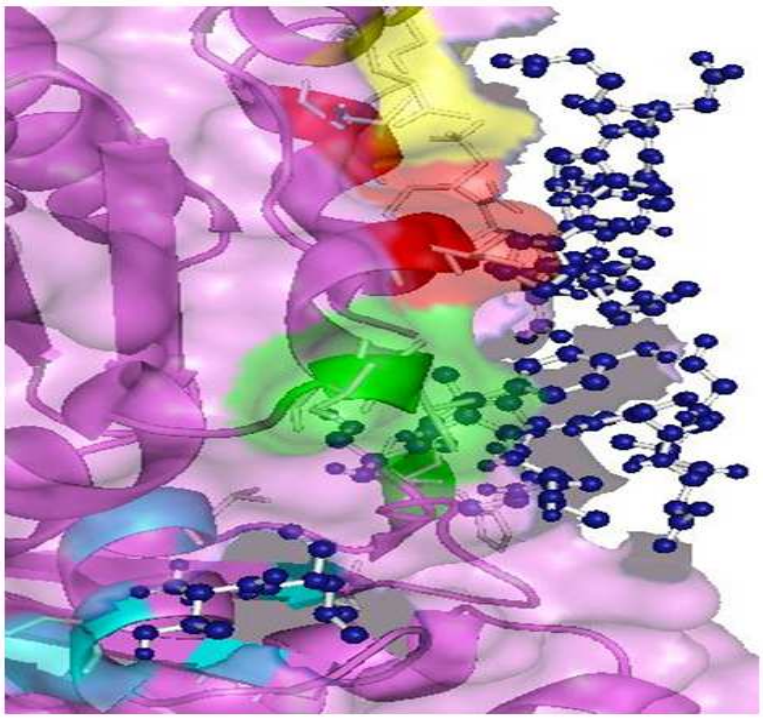


Fig. 6. *A.niger* RNase represented in ball and stick form, in which carbon-density blue, hydrogen-white, nitrogen-blue, oxygen-red and sulfur-orange color. Protein-protein docking interaction of *A.niger* RNase represented in rainbow and *L. donovani* actin in vacuum electrostatic light blue white color

Table 1. PROCHECK and ERRAT

	PROCHECK							
	Ramachandran Plot Quality (%)				Goodness Factor			
	Most favored	Additional allowed	Generously allowed	Dis-allowed	Dihedral	Covalent	Overall	ERRAT Score
RNase	100	0	0	0	-0.05	0.37	0.13	84.615
3D3Z	86.5	13	0	0.5	-0.2	0.28	-0.01	97.391
Actin	95.4	4.6	0	0	0.11	-0.14	0.02	84.239
1YAG	94.4	5.6	0	0	0.18	0.45	0.3	98.039

Table 2. WHAT IF stereochemical quality evaluation

Structure	Average[a] package quality	Rotamer Normality[b] X_1X_2	Backbone Confornatiom	Bond length[c]	Angle[d]
RNase	3.124	-0.248	-2.362	0.919	1.300
3D3Z	0.785	0.202	-0.645	0.775	0.775
Actin	-0.453	-0.364	-0.809	0.910	1.212
1YAG	-0.055	-2.248	-0.282	0.469	0.705

[a]The average quality of 200 highly refined X-ray structures was -0.5±0.4 [b]The behaviour of the distribution is much that a Z-score below -2 (2 standard deviations way from the average) is poor and a Z-score of less than -3 is of concern; positive is better than average. [c]RMSD Z-score should be close to 1.0 [d]RMSD Z-score, more common values are around 1.55

Table 3. Protein-protein docking results from HEX server

Receptor Protein	Lead Protein	Cluster	Solution	RMSD from reference Structure (Å)	Etotal (kcal/mol)	Docked energy (kcal/mol)
L. donovani actin	*A.niger* RNase	1	1	0.854	-1.789	-2.262
		2	2	0.689	-1.925	-7.002
		3	3	0.856	-1.879	-9.154
		4	5	0.431	-1.821	-6.452
		5	19	0.789	-1.724	-7.986
		6	17	0.921	-1.652	-6.285
		7	11	0.402	-1.902	-9.323
		8	22	0.599	-1.652	-8.930
		9	24	0.612	-1.706	-3.526
		10	25	0.784	-1.699	-7.854

3.4. Protein-protein Docking

In order to understand the inhibition mechanism of *A.niger* RNase on *L. donovani* actin, primary docking calculations were performed with HEX. The goal of the initial stage of docking was to generate as many near-native complex structures (hits) as possible. The generated PDB file was analyzed for their binding conformations. Analysis was based on Etotal or free energy of binding, lowest docked energy and calculated RMSD values. For each approach, the number of hits, the RMSD value of the best hit (with the lowest RMSD) based on shape complementarity are listed in **Table 3**. The results obtained from protein-protein docking algorithms were satisfactory. The total clusters of docking conformations, with the top 30 docked molecules showed negative binding energies. Cluster 1 show the docked energy and RMSD values to be -2. 262 kcal/mol and 0.854 Å, respectively. Among all docking clusters, rank 07 i.e., solution 11 gave the best predicted binding free energy of -1.902 kcal/mol with RMSD value of 0.402 Å. *A.niger* RNase docking revealed that the amino acids Thr-1, Leu-2, Asp-3, Ser-4, Tyr-5, Thr-6, Ala-7, Leu-8, Pro-15, Ser-16 and Glu-17 played vital role to bind the Vla-140, Leu-141, Ser-145, Ile-152, Pro-165, (Highlighted with cyan color), Pro-173, His-174, Thr-350 and Thr-351, (Highlighted with green color), Glu-362 and Ser-366, (Highlighted with yellow color), Ser-369, Ile-370, Asn-373, Lys-374 and Thr-355 (Highlighted with red color) of *L. donovani* actin **Fig 6**. Our *in-silico* experiments demonstrated that *A.niger* RNase binds *L. donovani* actin and also is itself inhibits its function and thus may act as a drug. Cumulatively, the evidence leads us to propose that cell-surface actin could be the target for RNase in leishmanial cells.

4. DISCUSSION

The BLASTp result of *A.niger* RNase showed 100% sequence similarity to ACTBIND (PDB ID: 3D3Z) where as in the case of *L. donovani* actin sequence similarity was 99% with structure of the yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG). Thus two structures ACTBIND (PDB ID: 3D3Z) (Gonzalez and Almog, 2008) and structure of the yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG) were selected as templates (Schutt *et al*., 1993) for *A.niger* RNase and *L. donovani* actin, respectively. The most significant step in homology modeling process is to obtain the correct sequence alignment of the target sequence with the homologues.

Among the available potential templates, crystal structure of ACTBIND (PDB code: 3D3Z, resolution 1.7 Å, R-value 0.191 (obs) and R-Free-0.231) (Gonzalez and Almog, 2008) was selected as the template structure to construct molecular model of the *A.niger* RNase. The same procedure was applied for modeling the 3D structure of *L. donovani* actin based on the structure of the yeast actin-human gelsolin segment 1 complex (PDB code: 1YAG, resolution 1.90 Å, R-value 0.193 obs) (Schutt *et al*., 1993).

Therefore, *A.niger* RNase may compete with angiogenin for cell-surface actin and, in this manner, blocks the formation of the actin-angiogenin complex required for leishmanial cell organization and angiogenesis in developing neoplastic tissue.

5. CONCLUSION

In this study, we have developed very high accurate 3D models of *L. donovani* actin protein and *A.niger* RNase by using the templates ACTBIND (PDB ID:

3D3Z) and structure of the yeast actin-human gelsolin segment 1 complex (PDB ID: 1YAG), respectively in Modeller 9v8. This model has been qualified using several validation methods, including PROCHECK, ERRAT, WHAT-IF, PROSA, 2003 and VERIFY-3D. All evidences suggested that the geometric quality of the backbone conformation, the residue interaction, the residue contact and the energy profile of the structure was well within the limits established for reliable structures. The protein-protein docking study has been elucidated for the purpose of finding antileishmanial property of *A.niger* RNase. The interaction energy of docking between the *A.niger* RNase and *L. donovani* actin was calculated and analyzed using the HEX server. The efficient binding of *A.niger* RNase and *L. donovani* actin revealed that the proteins could form hydrogen bond networks involving active amino acid residues. Several amino acid residues including Thr-1, Leu-2, Asp-3, Ser-4, Tyr-5, Thr-6, Ala-7, Leu-8, Pro-15, Ser-16 and Glu-17 were identified to exclusively contributive to the binding of *A.niger* RNase to Val-140, Leu-141, Ser-145, Ile-152, Pro-165, Pro-173, His-174, Thr-350, Thr-351, Glu-362, Ser-366, Ser-369, Ile-370, Asn-373, Lys-374 and Thr-355 of *L. donovani* actin. The generated homology model is expected to be useful for the structure-based drug design against leishmaniasis.

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