Chapter

rRNA Platform Technology for Drug Discovery Methods for Identifying Ligands That Target Plasmodium RNA Structural Motifs

Harrison Ndung'u Mwangi and Francis Jackim Mulaa

Abstract

Determining the structure of the *P. falciparum* 40s leads to better understanding of the structural basis for its protein-synthesizing roles in the cell. This enables researchers in the field of drug development to run *in silico* ligand screening experiments using the solved *P. falciparum* 40S structure as a target against a library of potential anti-malarial compounds. Drug leads identified through this method can lead to further biochemical and *In vitro* binding studies with the ultimate goal of developing new class of anti-malarial drugs. The use of structure prediction and modeling technologies in this study dramatically reduces the time it takes from target identification to drug lead determination. Furthermore, very many compounds that were previously incapable of being experimentally tested can now be tested *in silico* against the generated structure. Owing to the increasing utility of bioinformatics and three dimensional structural modeling software, one can accurately build physical models solely from sequence data by unwrapping the information therein on probable motif sites capable of being anchored onto available compounds or aptamers.

Keywords: *P. falciparum*, Ribosome, 40S subunit, *In silico*, structure determination, Dynamic simulations, docking

1. Introduction

Ribosomes are cellular organelles found in the cytoplasm and primarily responsible for protein synthesis in the cell. Ribosomes' were first observed as dense particles or granules under an electron microscope [1]. The Eukaryote ribosome is a large complex (about 2.6 MDa) molecular machine composed of rRNAs and proteins [2–4]. In the past few years, a combination of X-ray crystallography, NMR spectroscopy and Cryo-electron microscopy has provided new data on the structure of ribosomes [5]. The Eukaryotic ribosome (80S) comprises of two subunits, a large subunit (60S) and small subunit (40S). The ribosome plays a major role during translation of RNA to the various proteins they code for. The process of translation occurs when the message contained in mRNA is decoded and the respective amino

acids synthesized into a growing polypeptide chain which eventually folds into a three dimensional functional structure. Protein synthesis is critical for cell viability, hence highlighting the importance of the ribosome in the cell [6].

1.1 Eukaryotic ribosome

A eukaryote ribosome where Plasmodium species falls is designated as 80S and contains two subunits. The smaller subunit 40s is comprised of 18 s rRNA and 33 proteins whereas the large subunit 60S (**Figure 1**) is comprised of the 28S, 5S and 5.8S rRNA and 49 proteins [7–9].

X-ray crystallography and Cryo-electron microscopy methods have been used to solve the three dimensional structures of the ribosome with or without complexed cofactors examples be tRNA, mRNA among other macro molecules successfully [10]. The very first models of eukaryotic ribosome's at resolution between 6.1 and 15 Å were provided by Cryo-electron microscopy, revealed the location and the shapes of the RNA expansion segments and indicated the position of additional protein moieties [11–13]. Later, a crystalline structure of the complete eukaryotic ribosome from *Saccharomyces cerevisiae* was determined at 4.15 Å and later 3.0 Å resolutions [9, 14]. These structures with more clarity gave more insights on understanding the process of translation which captured the ratcheted states of the ribosome which had been postulated over 40 years [15, 16]. Recently the overall crystal structure of the eukaryotic ribosome of *S. cerevisiae* obtained revealed basic architectural similarity, but the larger assembly compared with the prokaryotic counterpart [14].

In addition this structure shows the E-site, A-site, the ribosomal proteins of both the 60S and the 40S subunits together with the expansion segments which gave more knowledge about eukaryotic protein synthesis process. This followed through earlier studies that were done and showed both interfaces of the 60S and 40S subunits views with numbered bridges [17].

1.2 18S rRNA structure

RNA molecules are polymers of nucleotides comprised of 3′-5′ phosphor-diester linked ribose sugars attached to the four bases, pyrimidines: cytosine and uracil and

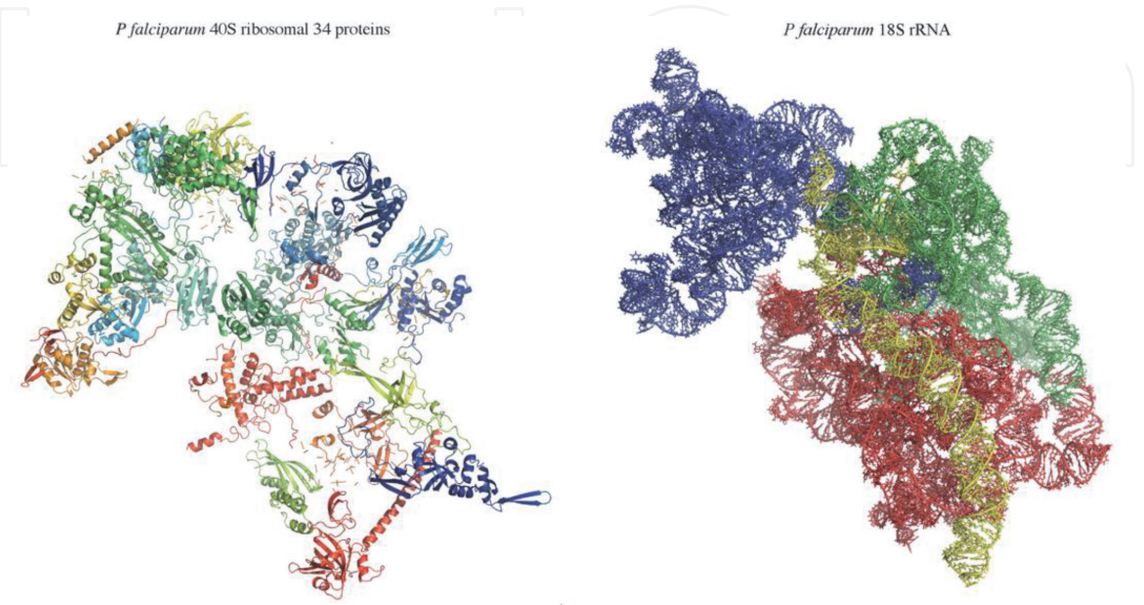

Figure 1.
A graphical presentation of the components of the 40S ribosomal subunit of Plasmodium falciparum.

purines: adenine and guanine in contrast with the DNA [18]. RNA is single stranded containing ribose sugars and uracil base in place of thymine.

While the G-U wobble pair is somewhat different in shape. Base pairing occurs in both DNA and RNA using the hydrogen bonds formed this pairing patterns are governed by Watson and crick rules [19], where adenine complements uracil in RNA (thymine in DNA) and guanine with cytosine in both known as conical base pairing. In places where this pairing does not happen it's known as non-Watson crick structures among these are the sheared GA, GA imino, AU reverse Hoogsteen, and the GU and AC wobble pairs (**Figure 2**) [19, 20]. Determining the RNA secondary structure is the first step of understanding its mechanism of action which is defined by the canonical base paring [21]. The secondary structure of the RNA can adopt elements such as internal loops, mismatches bulges, multi-branched junctions, hairpins and pseudo knots (**Figure 3**).

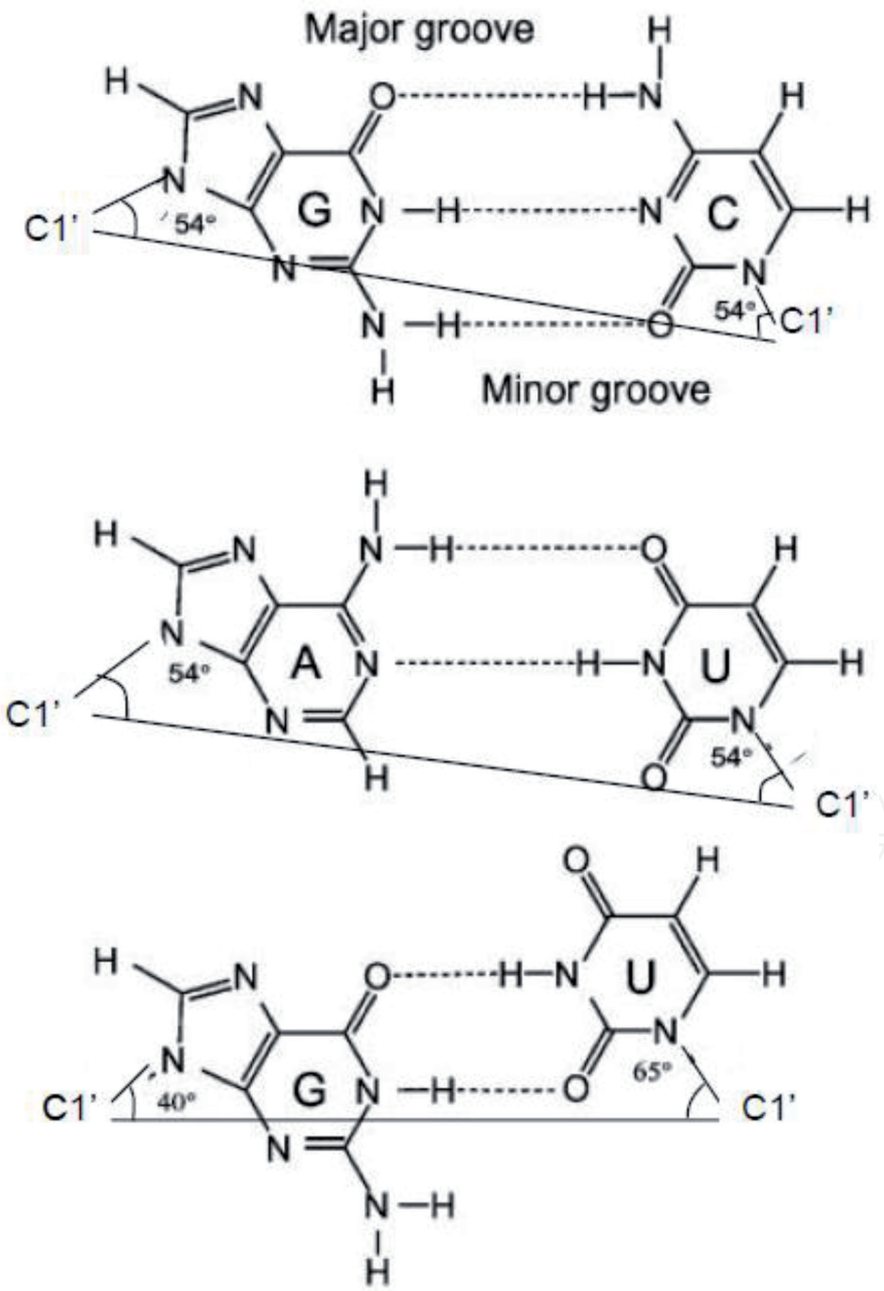

Figure 2.
Watson–Crick G-C and A-U base pairs with a similar angle of ~54. The G-U "wobble base pair" is also shown [9, 18].

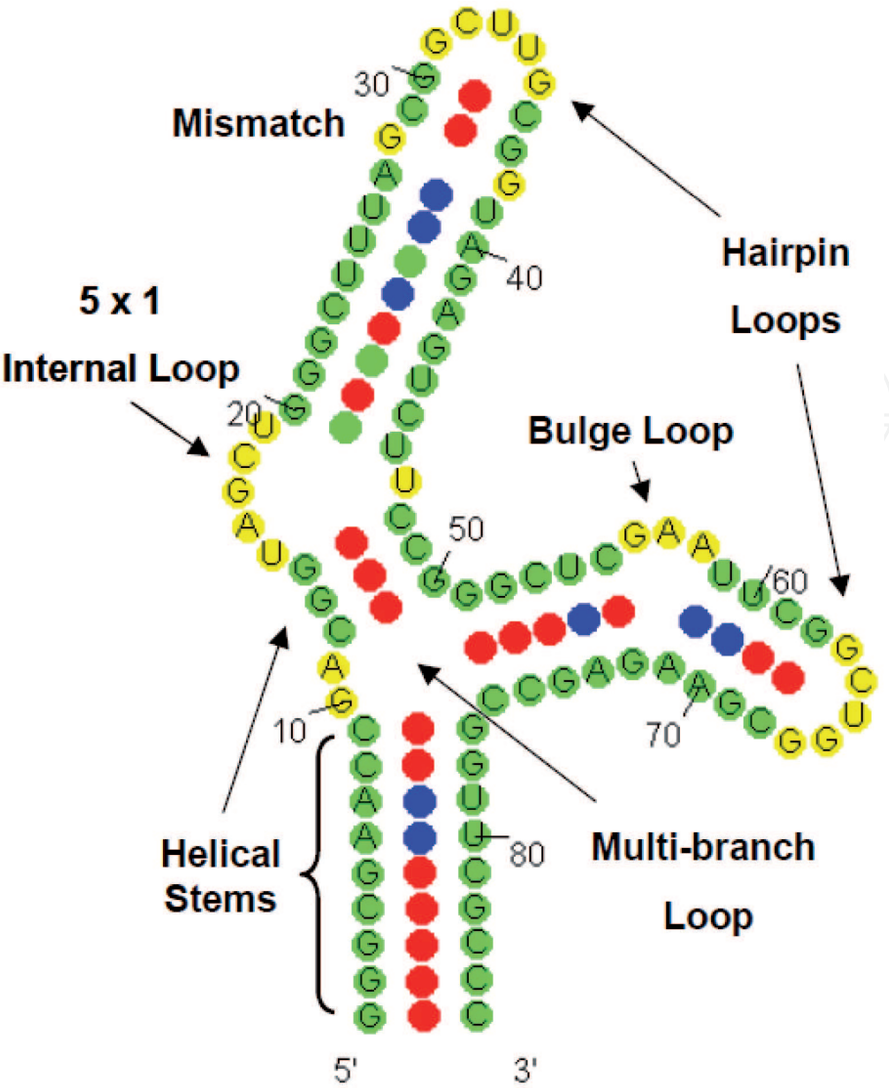

Figure 3.
RNA secondary structure motifs showing representation of Watson crick base paring [9, 18].

Presence of these diverse motifs that can be adopted by the RNA makes it play its functional roles such as recognition, interaction, metal binding and other enzymatic activities [20]. The 18S fold primarily defines the eukaryotic 40S subunit structure which can be divided into features of the small ribosomal subunit including the head, platform, body, beak, shoulder, right foot and left foot (**Figure 4A**) [22]. The secondary structure of the eukaryotic ribosome forms a structure with four major domains which are named according to the region of the sequence from 5′ to the 3′ end. They are 5'major, central, 3'major, and finally 3'minor (**Figure 4B**).

Figure 2 Watson–Crick G-C and A-U base pairs with a similar angle of ~54. The G-U —Wobble base pair‖ is also shown [20]. While the G-U wobble pair is somewhat different in shape. **Figure 3** RNA secondary structure motifs showing representation of Watson crick base paring [20].

The 18S rRNA is composed of a region homologous to the prokaryotic 16S rRNA with several eukaryotic specific ESs [23] as shown in **Figure 5**. The ESs helical elements in eukaryotes display variable lengths; however their architecture is found to be preserved [22]. Both segments of ES3 are located on the 5′ domain, ES6 and ES7do form insertions in the central domain, and ES9 and ES12 are found in the 3′ major and 3'minor domain of the 18S RNA, respectively [22].

Eukaryotic 18S rRNA ES6, is the longest consisting of ~250 nucleotides which form five helices that replace the bacterial helix 21. ES6 is inserted between h20

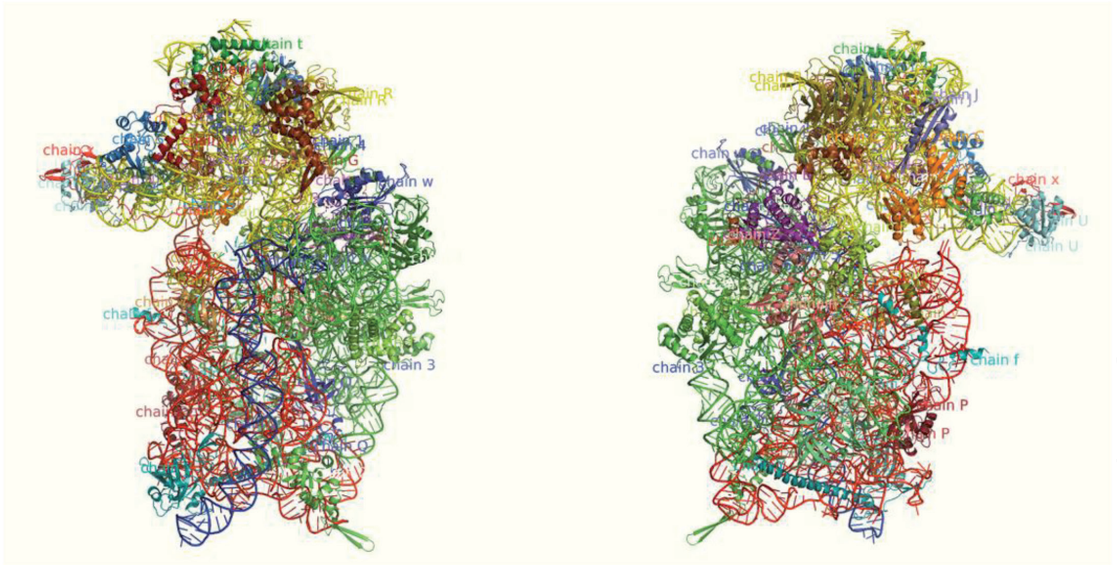

Figure 4.
Architectural tertiary structure of Plasmodium falciparum *40S front and back view. The 18S rRNA is colored differently depending with domains (5'major –red, central-yellow, 3'major-blue and 3'minor- green). Also shown are the 40S ribosomal 34 proteins of* Plasmodium falciparum *interacting with the 18S rRNA to make the total subunit.*

and h22 in the lower back region of the 40S body. A, B, C, and D helices form a large portion of the back of the 40S body and their structure differs considerably from a previous model [24]. C and D Helices are located at an equivalent position to bacterial h21. They stretch across the back of 40S and are buried underneath helix A.ES6B apical loop region is exposed and disordered in the structure, which makes it prone to chemical modification and cleavage by nucleases, as previously observed [25]. The loop region ES3B forms a base pair with helix ES6E loop region which yields to an extended helix which leads to projection from the center of the back toward the left foot of the 40S. Earlier demonstration by computational and biochemical experiments showed this rather unusual quaternary interactions.

The left foot along with ES6E is formed when the ES3B apical region packs against ES3A. ES3 and ES6 quaternary eukaryotic interaction, together with several proteins form a new domain, responsible for more prominent left foot features of the 40S and a broader back. Situated directly below the bleak is helix h16, which is shifted relative to the position in bacteria by as much as 40 Å [25].

In the 40S crystal structure the position of h16 is consistence with those observed in the solution by Cryo-EM of the empty yeast 40S, the 40S-eIF1 or40S-eIF1A complex [26], and the canine and *Thermomyces lanuginosus* 80S [24, 27]. Formation of a connection between the head and the body of the 40S subunit involves h16 upon binding of the initiation factors eIF1 and eIF1A, which might point to a role of this helix in initiation [26].

1.3 RNA as a drug target

The pharmaceutical industries together with the researches have always forecast their efforts more on protein, rather than the nucleic acids as suitable targets of drugs. But the acceleration and advances of studies on the RNA synthesis, structure determination and therapeutic target identification has blown open the question of using RNA as a drug target a very genuine area [28]. In this age due to the wealth of the three dimensional structure of the RNA in various repositories, it is possible they could be used for drug design by observing the target design. It has been a

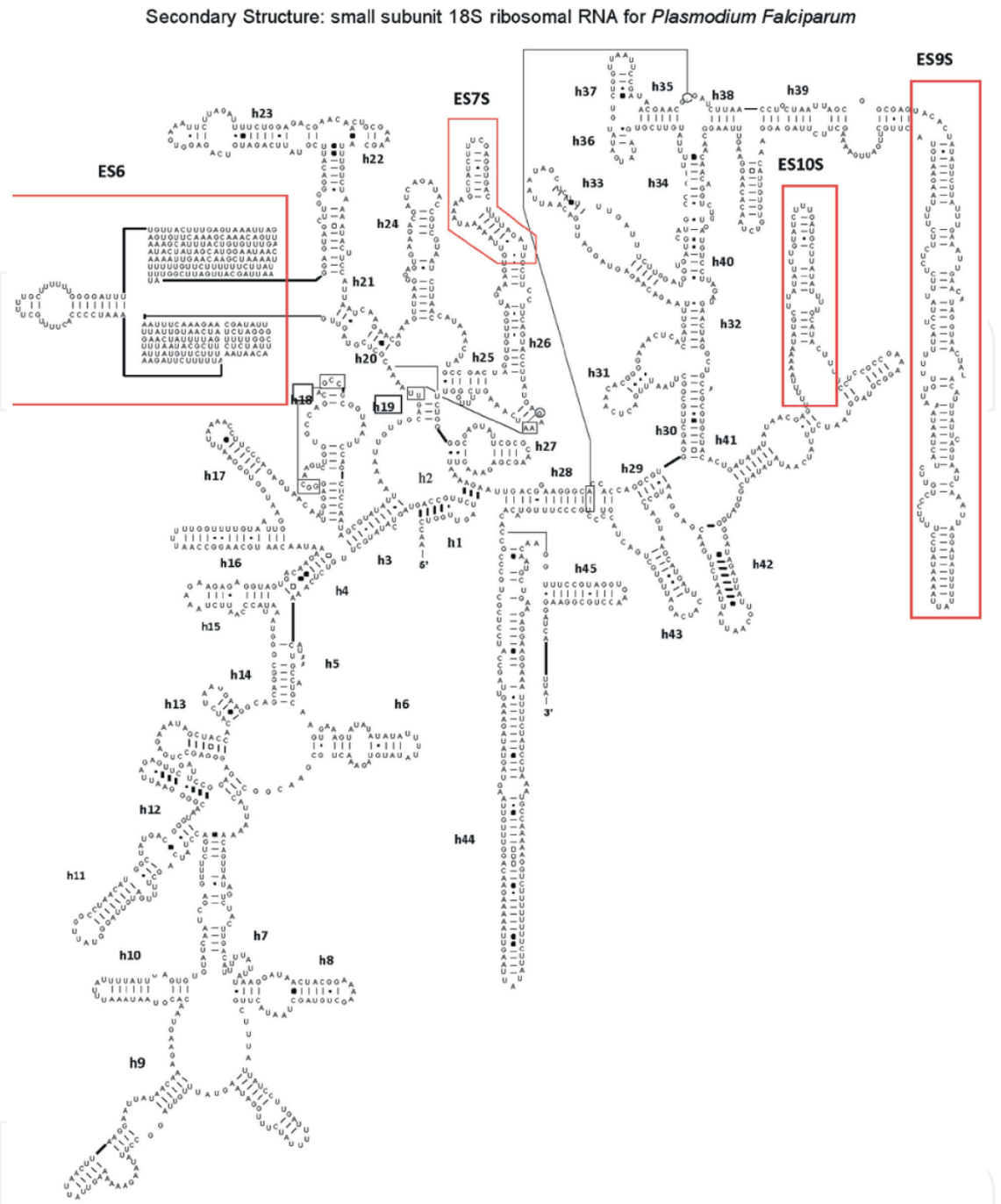

Figure 5.
18S rRNA secondary structure of Plasmodium falciparum *showing the four expansion segment regions (ES) located at the central and 3'major domain [9].*

difficult task in the past to obtain a three-dimensional structure for large RNAs using the crystal and magnetic resonance experimental techniques, which was just restricted to very small fragments [28–30] but newer techniques and more input to the older experimental methods are leading to accurate much larger structures. The RNA on its chemical basis does not show a promising drug target in that it's made up of four different planer bases and negatively charged nucleus [29, 31]. But upon the RNA adopting its conformational architecture, that shows the presence of cavities and pockets which could bind to shape specific rather than sequence specific molecules [31]. The RNA cavities compels the phosphate groups to be in close proximity, that lead to an intensified importance of tightly bound water molecules, electrostatic forces and ions, magnesium divalent ions in particular that can be partly dehydrated [30, 32]. Presence of non-Watson Crick pairs and bulged residues

leads the formation of pockets and enlarged grooves that infer function through assembly of existing RNA motifs which are architecturally clearly diverse [30, 33]. The secondary structure of the RNA consist regular double stranded helices that gives it most of its energy content which in turn folds to subsequent three dimensional structure free energy content of between 5-10kcalmol [34]. This gives a Nano molar range binding constant which can be achieved for a small molecule usable to compete with the final step of RNA folding [30, 35]. From the discussed above projections with a number of ways of inserting non Watson crick pairs within helices is very limited leading to the restriction in the number of RNA motifs that are picked as drug target [30]. RNA motifs appear as decreasing size placed one inside the other, with the smaller motif associated with the larger motif [36, 37]. It has been shown that some antibiotics bind structurally in different regions of RNA molecules in the ribosome, such as streptomycin binding to the shallow groove, hygromycin binds to the deep groove of the helix, aminoglycosides at three adenine bulges and macrolides in the tunnel of the nascent polypeptide chain [8, 38–40].

Streptomycin interacts with only phosphate groups of many [40]. In conclusion there are various other ways that ligands interact with the RNA that are not known yet and the growth of a rich three dimensional structural of both RNA and ligands leads to newer technique of drug discovery that may include binding and docking experiments of the structures to obtain newer and stronger intervenes.

2. Methodology

Functional RNA elements have specific RNA sequences and structures that work together as a functional unit. From the perspective of bioinformatics, homology-based searching for both conserved sequences and secondary structures is effective at finding structured RNA motifs [33]. These strategies to determine RNA structures provide the means to investigate the potential of these RNA motifs as drug targets. Although drug development can be accomplished without knowing how a compound works in the cell, hit-to-lead optimization (during which small molecule hits from a high-throughput screen undergo optimization in order to identify promising lead compounds) is greatly facilitated if the target is known [34].

Here, we present a set of in-silico based methods for developing reliable atomic level 3D-models of RNA structures efficiently and cheaply, using sequence information, to accelerate the drug discovery process for plasmodium. Our methodology employs molecular modeling and structure based drug design approach in which we use the 3D experimental ribosome structures information of Plasmodium to identify RNA motifs that could be potential drug targets. Using the information of the rRNA structure and movement we have identified and docked a set of promising anti-infective-like compounds that target biologically functional ribosomal RNA motifs that could be potential drugs against malaria.

2.1 RNA tertiary structure prediction

To determine the spatial structure of RNA, researchers can use experimental techniques, such as bio-crystallography or NMR spectroscopy. However, the experimental techniques, are tiresome, expensive, and require specialized equipment [41]. An alternative to the experimental techniques are computer modeling methods. Although the computer modeling methods are not as accurate as mentioned above experiments, they can be successfully used to investigate the function and mechanism of action of the RNA molecules [42, 43].

Therefore, there is a need for computational methods that can provide reliable models of RNA structures efficiently and cheaply, using only information on a nucleotide sequence [44]. The goal of computational structural bioinformatics is not to replace experimental techniques, but to compliment them especially when the answer for scientific questions are beyond their reach [45]. Unfortunately, despite the fact that computational methods are being continuously improved, they not always predict the correct structures of RNA [44].

The secondary structure determination (or prediction) is often the starting point for the spatial (3D) structure determination of RNA [46]. Methods for predicting tertiary structure of RNA are generally represented in two categories: The first category comprising of the methods that are based on the laws of physics known as ab initio modeling and the second category comprising of the methods based on experimental data.

Methods based on experimental data are further divided to either those that extrapolate knowledge of experimentally solved structures known as de novo modeling and the methods that extrapolate the fragments of already solved structures which include assembly-based methods, comparative or homology modeling and manual building structures based on figments [47–51].

Our methodology focused on using the homology and de novo prediction modeling methods of RNA 3D structure development to produce the 3D structure of the ribosomal RNA (rRNA) of the plasmodium. The implementation was done using ModeRNA [50], RNA123 [20], SimRNA [52, 53]. Any refinement was done by QRNAS software [54].

2.1.1 Modeling of the rRNA model

The ribosomal core model is used as an anchor point for modeling the expansion segments and variable regions. RNA sequences from the structure based sequence alignment were loaded in FASTA format and the cleaned template structure also loaded. The data is submitted for structure modeling as shown by **Figure 6**.

The modeling proceeds by getting a sequence which is obtained from atomic coordinates in one-letter-code, getting a secondary structure by extracting the 2D structure from 3D and return it in the Vienna format and finally analyzing the geometry to check whether the residues of the structure have any unusual features like strange bond and angle values.

2.1.2 Structure evaluation

The WebRASP server is used to compute energy scores for assessing the stability of the modeled RNA structure. The server receives as input a PDB file containing the atomic coordinates of the RNA structure and calculates the energy profile and total energy score of the molecule. The server displays the results graphically and the visualization can be modified by the user. The server relies on RASP [55] for the calculation which is an all-atom knowledge-based potential assessment for scoring of RNA 3D structures based on distance-dependent pairwise atomic interactions.

Further validation is done using MolProbity [56, 57] which is a structure validation server used for all-atom contact analysis. The web service provides broad-spectrum model diagnosis and repair. It evaluates the model quality at both the global and local levels. It relies heavily on the power and sensitivity provided by optimized hydrogen placement and all-atom contact analysis, complemented by updated versions of covalent-geometry and torsion-angle criteria.

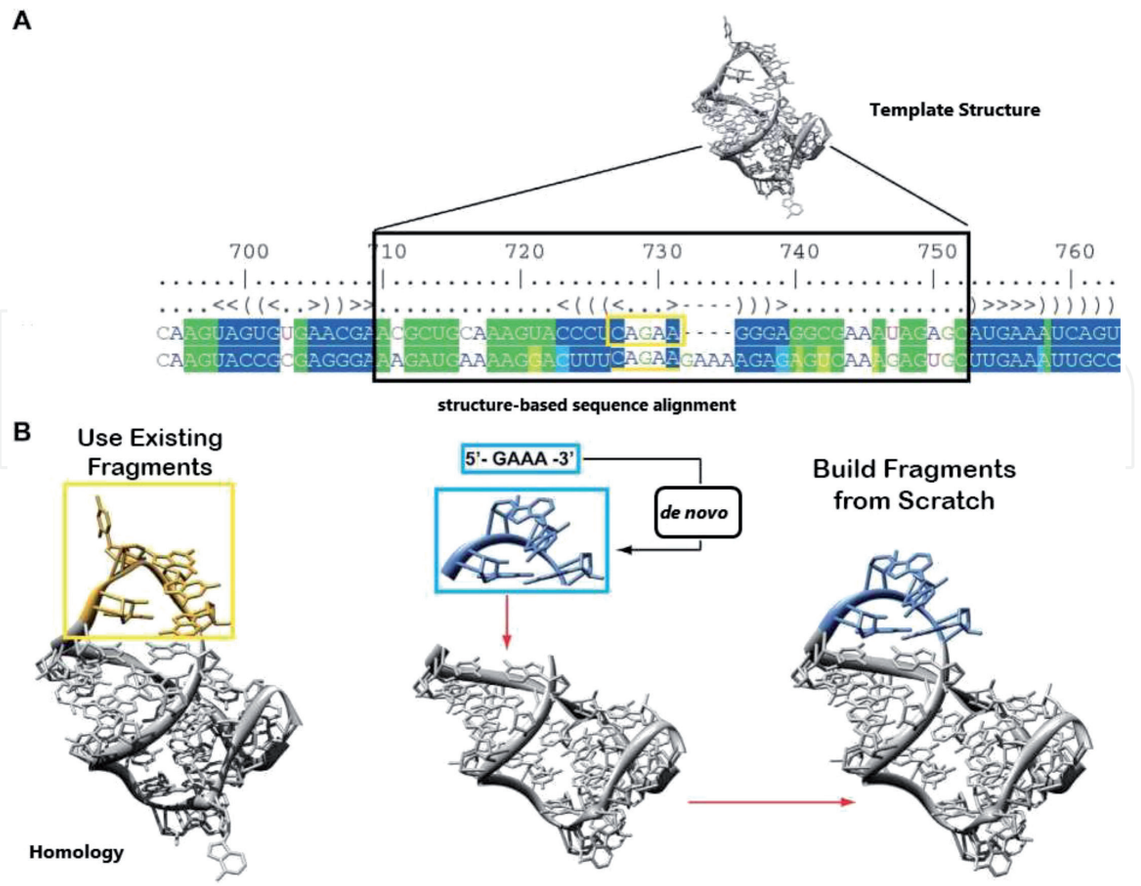

Figure 6.
The schematic diagram of RNA modeling.

2.2 Molecular dynamics simulation

SimRNA [52, 53] was used to analyze conformational landscapes and identify potential alternative structures using the default parameters; 500 simulation steps and 1% of the lowest energy frames of the input subjected to clustering. The software employs a statistical potential to approximate the energy and identifies conformations that correspond to biologically relevant structures.

2.2.1 Visualization and structure analysis

The sequence based and structure-based alignments are visualized using GNU emacs with RALEE [58] and Sublime text3 [59]. The secondary structures are visualized in Varna [60] and xRNA. The 3D model is visualized using USF Chimera [61, 62] and PyMol [63]. Video representations of the model are made using BIOVIA Discovery Studio [64].

2.3 Identification of rRNA motifs

As functional components in three-dimensional conformation of an rRNA, structural motifs provide an easy way to associate the molecular architectures with their biological mechanisms.

One major computational approach for studying RNA structural motifs is to search homologous instances of known motifs by using comparative methods. Traditionally, the motifs are modeled with their 3D geometric features, such as backbone conformations or torsion angles. Although a number of typical tools

primarily rely on the 3D atomic coordinates and do perform well for some simple motifs, they may not work for complex ones since the underlying computational methods are too rigid to identify the flexible variations in structures. Using thin formation we integrate pairwise interactions as constraints into the screening for RNA structural motifs besides 3D information, using the FR3D platform [65]. However, as the most critical character of RNAs, the base–base interactions should be used as key factors in the assessment of structural discrepancy directly. Based on this idea, we use RNA Motif Scan to search new motif candidates that share non-canonical base–base interaction patterns with the query [66, 67].

2.4 Structure based molecular docking

Molecular docking is also referred to as small molecular docking is a study of how two or more molecular structures, for instance, drug and catalyst or macromolecule receptor, match along to be a perfect fit [68]. The study employed both the Shape complementarity approach using PatchDock [69].

Shape complementary approach was successful in producing the docking results from compound in the Malaria Pathogen Box.

3. Conclusions

Malaria is a disease spread by the female anopheles mosquito that contains protozoan organisms of the plasmodium genus that actually cause malaria [13]. These thrive in tropical and subtropical areas which predominant in Africa. According to WHO Sub-Saharan Africa carries a disproportionately high share of the global malaria burden.

Malaria is transmitted by the anopheles female mosquito that carries *the P falciparum* that causes malaria. There are about 100 types of plasmodium parasites which can infect a variety of species. Scientist have identified five types that specifically affect humans but of all *P. falciparum* stands out. *P falciparum* found worldwide in tropical and rural ranges, however predominately in Africa. An expected 1 million individuals are killed by this strain each year. The strain can multiply quickly and can adhere to blood vessels in the brain, bringing causing rapid onset of severe malaria including cerebral malaria [9].

The rRNA is a very core component of any cell for the *P falciparum* the 18S rRNA is where we draw our attention to, this is because even after the *P falciparum* undergoes mutation the 18S rRNA of it rarely undergoes any change thus if targeted there will not mutate to resist the drugs. In designing drugs that only attack the rRNA of the *P falciparum* not the proteins therefore we are able to make drugs that are more effective and have a higher efficacy. By docking a large library of compounds into a high-resolution structures of the target receptor 18S rRNA, fewer compounds typically need to be experimentally screened to identify compounds that are active against the target. These rRNA are the most abundant species of RNA in the living cells. They are the largest component of the ribosomes; large RNA-protein particles that form sites for synthesizes proteins and form the bulk of it. rRNAs have evolved as a hub of protein biosynthesis in all living cells performing both catalytic, regulatory and organizational roles [70, 71].

This spurred significant progress in the understanding biology of ribosomes. For example; the translational mechanism of the ribosome and the mode by which the function of the ribosome is altered by antibiotic inhibitors [4, 72]. These results have stimulated new interest in extending our understanding to the more complicated eukaryotic ribosome.

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As we begin to get more insights in the working of the prokaryotic ribosomes, the question we are also seeking to answer is "How does the complex eukaryotic ribosome assemble inside living cells? A number of approaches are beginning to address this question. The approaches comprise of a combination of genetic, biochemical, and structural approaches.

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References

[1] Frank J. The Ribosome Comes Alive. Israel Journal of Chemistry. 2010;50(1): 95-98. doi: https://doi.org/10.1002/ijch.201000010.

[2] Garrett R. Mechanics of the ribosome. Nature. 1999;400(6747): 811-812. doi:10.1038/23573.

[3] Noller H, Lancaster L, Zhou J, Mohan S. The ribosome moves: RNA mechanics and translocation. Nature Structural & Molecular Biology. 2017;24:1021-1027. doi:10.1038/nsmb.3505.

[4] Ramakrishnan V. Ribosome Structure and the Mechanism of Translation. Cell. 2002;108(4):557-572. doi:https://doi.org/10.1016/S0092-8674(02)00619-0.

[5] Frank J, Heagle AB, Agrawal RK. Animation of the Dynamical Events of the Elongation Cycle Based on Cryoelectron Microscopy of Functional Complexes of the Ribosome. Journal of Structural Biology. 1999;128(1):15-18. doi: https://doi.org/10.1006/jsbi.1999.4138.

[6] Moore SD, Sauer RT. The tmRNA System for Translational Surveillance and Ribosome Rescue. Annual Review of Biochemistry. 2007;76(1):101-124. doi:10.1146/annurev.biochem.75.103004.142733.

[7] Ben-Shem A, Garreau de Loubresse N, Melnikov S, Jenner L, Yusupova G, Yusupov M. The Structure of the Eukaryotic Ribosome at 3.0 Å Resolution. Science. 2011;334(6062): 1524. doi:10.1126/science.1212642.

[8] Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Hartsch T, Wimberly BT et al. Crystal Structure of an Initiation Factor Bound to the 30S Ribosomal Subunit. Science. 2001;291(5503):498. doi:10.1126/science.1057766.

[9] Mwangi, Harrison Ndung'u., Peter Wagacha, Peterson Mathenge, Fredrick Sijenyi, and Francis Mulaa. "Structure of the 40S ribosomal subunit of *Plasmodium falciparum* by homology and de novo modeling." *Acta pharmaceutica sinica B* 7, no. 1 (2017): 97-105..

[10] Ban N, Nissen P, Hansen J, Moore PB, Steitz TA. The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution. Science. 2000;289(5481):905. doi:10.1126/science.289.5481.905.

[11] Spahn CMT, Jan E, Mulder A, Grassucci RA, Sarnow P, Frank J. Cryo-EM Visualization of a Viral Internal Ribosome Entry Site Bound to Human Ribosomes: The IRES Functions as an RNA-Based Translation Factor. Cell. 2004;118(4):465-475. doi:10.1016/j.cell.2004.08.001.

[12] Spahn CMT, Beckmann R, Eswar N, Penczek PA, Sali A, Blobel G et al. Structure of the 80S Ribosome from Saccharomyces cerevisiae—tRNA-Ribosome and Subunit-Subunit Interactions. Cell. 2001;107(3):373-386. doi:https://doi.org/10.1016/S0092-8674(01)00539-6.

[13] Becker T, Bhushan S, Jarasch A, Armache J-P, Funes S, Jossinet F et al. Structure of Monomeric Yeast and Mammalian Sec61 Complexes Interacting with the Translating Ribosome. Science. 2009;326(5958): 1369. doi:10.1126/science.1178535.

[14] Ben-Shem A, Jenner L, Yusupova G, Yusupov M. Crystal Structure of the Eukaryotic Ribosome. Science. 2010;330(6008):1203. doi:10.1126/science.1194294.

[15] Spirin AS. The Second Sir Hans Krebs Lecture. European Journal of Biochemistry. 1969;10(1):20-35.

doi:https://doi.org/10.1111/j.1432-1033.1969.tb00651.x.

[16] Bretscher MS. Polypetide chain termination: An active process. Journal of Molecular Biology. 1968;34(1):131-136. doi:https://doi.org/10.1016/0022-2836(68)90239-8.

[17] Yusupov MM, Yusupova GZ, Baucom A, Lieberman K, Earnest TN, Cate JHD et al. Crystal Structure of the Ribosome at 5.5 Å Resolution. Science. 2001;292(5518):883. doi:10.1126/science.1060089.

[18] Sijenyi, Fredrick, Pirro Saro, Zheng Ouyang, Kelly Damm-Ganamet, Marcus Wood, Jun Jiang, and John SantaLucia. "The RNA folding problems: different levels of sRNA structure prediction." In *RNA 3D structure analysis and prediction*, pp. 91-117. Springer, Berlin, Heidelberg, 2012.

[19] Watson JD, Crick FHC. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. Nature. 1953;171(4356):737-738. doi:10.1038/171737a0.

[20] Sijenyi F, Saro P, Ouyang Z, Damm-Ganamet K, Wood M, Jiang J et al. The RNA Folding Problems: Different Levels of sRNA Structure Prediction. In: Leontis N, Westhof E, editors. RNA 3D Structure Analysis and Prediction. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 91-117.

[21] Mathews DH, Turner DH. Prediction of RNA secondary structure by free energy minimization. Current Opinion in Structural Biology. 2006;16(3):270-278. doi:https://doi.org/10.1016/j.sbi.2006.05.010.

[22] Rabl J, Leibundgut M, Ataide SF, Haag A, Ban N. Crystal Structure of the Eukaryotic 40S Ribosomal Subunit in Complex with Initiation Factor 1. Science. 2011;331(6018):730. doi:10.1126/science.1198308.

[23] Cannone JJ, Subramanian S, Schnare MN, Collett JR, D'Souza LM, Du Y et al. The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics. 2002;3:2-. doi:10.1186/1471-2105-3-2.

[24] Taylor DJ, Devkota B, Huang AD, Topf M, Narayanan E, Sali A et al. Comprehensive Molecular Structure of the Eukaryotic Ribosome. Structure. 2009;17(12):1591-1604. doi:https://doi.org/10.1016/j.str.2009.09.015.

[25] Alkemar G, Nygård O. Secondary structure of two regions in expansion segments ES3 and ES6 with the potential of forming a tertiary interaction in eukaryotic 40S ribosomal subunits. RNA. 2004;10(3):403-411. doi:10.1261/rna.5135204.

[26] Passmore LA, Schmeing TM, Maag D, Applefield DJ, Acker MG, Algire Mikkel A et al. The Eukaryotic Translation Initiation Factors eIF1 and eIF1A Induce an Open Conformation of the 40S Ribosome. Molecular Cell. 2007;26(1):41-50. doi:https://doi.org/10.1016/j.molcel.2007.03.018.

[27] Chandramouli P, Topf M, Ménétret J-F, Eswar N, Cannone JJ, Gutell Robin R et al. Structure of the Mammalian 80S Ribosome at 8.7 Å Resolution. Structure. 2008;16(4):535-548. doi:10.1016/j.str.2008.01.007.

[28] Pearson ND, Prescott CD. RNA as a drug target. Chemistry & Biology. 1997;4(6):409-414. doi:https://doi.org/10.1016/S1074-5521(97)90192-7.

[29] Hermann T, Westhof E. Saccharide–RNA recognition. Biopolymers. 1998;48(2-3):155-165. doi:https://doi.org/10.1002/(SICI)1097-0282(1998)48:2<155::AID-BIP5>3.0.CO;2-I.

[30] Vicens Q, Westhof E. RNA as a Drug Target: The Case of Aminoglycosides. ChemBioChem. 2003;4(10):1018-1023. doi:https://doi.org/10.1002/cbic.200300684.

[31] Hermann T. Rational ligand design for RNA: the role of static structure and conformational flexibility in target recognition. Biochimie. 2002;84(9):869-875. doi:https://doi.org/10.1016/S0300-9084(02)01460-8.

[32] Velagapudi S, Seedhouse S, Disney M. Structure-Activity Relationships Through Sequencing (StARTS) Defines Optimal and Suboptimal RNA Motif Targets for Small Molecules. Angewandte Chemie (International ed in English). 2010;49:3816-8. doi:10.1002/anie.200907257.

[33] Batey RT, Rambo RP, Doudna JA. Tertiary Motifs in RNA Structure and Folding. Angewandte Chemie International Edition. 1999;38(16):2326-2343. doi:https://doi.org/10.1002/(SICI)1521-3773(19990816)38:16<2326::AID-ANIE2326>3.0.CO;2-3.

[34] Cundliffe E. Antibiotics as probes of ribosomal structure and function. In: Edwards DI, Hiscock DR, editors. Chemotherapeutic Strategy: Proceedings of the Symposium held on June 2-4 1982 at the World Trade Centre, London UK. London: Palgrave Macmillan UK; 1983. p. 65-78.

[35] Thomas JR, Hergenrother PJ. Targeting RNA with Small Molecules. Chemical Reviews. 2008;108(4):1171-1224. doi:10.1021/cr0681546.

[36] Grabow W, Andrews G. On the Nature and Origin of Biological Information: The Curious Case of RNA. Biosystems. 2019;185:104031. doi:10.1016/j.biosystems.2019.104031.

[37] Grabow WW, Zhuang Z, Swank ZN, Shea J-E, Jaeger L. The right angle (RA) motif: a prevalent ribosomal RNA structural pattern found in group I introns. Journal of molecular biology. 2012;424(1-2):54-67. doi:10.1016/j.jmb.2012.09.012.

[38] Brodersen DE, Clemons WMJ, Carter AP, Wimberly BT, Ramakrishnan V. Crystal structure of the 30 S ribosomal subunit from Thermus thermophilus: structure of the proteins and their interactions with 16 S RNA. J Mol Biol. 2002;316(3):725-768. doi:10.1006/jmbi.2001.5359.

[39] Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. Nature. 2000;407(6802):340-348. doi:10.1038/35030019.

[40] Brodersen DE, Clemons WM, Jr., Carter AP, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. The Structural Basis for the Action of the Antibiotics Tetracycline, Pactamycin, and Hygromycin B on the 30S Ribosomal Subunit. Cell. 2000;103(7):1143-1154. doi:10.1016/S0092-8674(00)00216-6.

[41] Hawkins AE, Fabris D. RNA Structure Determination by Structural Probing and Mass Spectrometry: MS3D. In: Leontis N, Westhof E, editors. RNA 3D Structure Analysis and Prediction. Nucleic Acids and Molecular Biology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 361-89.

[42] Klostermeier D, Hammann C. Optical spectroscopy and calorimetry. RNA Structure and Folding. 2015.

[43] Ding F, Dokholyan NV. Multiscale Modeling of RNA Structure and Dynamics. In: Leontis N, Westhof E, editors. RNA 3D Structure Analysis and Prediction. Nucleic Acids and Molecular

Biology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 167-84.

[44] Šponer J, Lankaš F. Computational Studies of RNA and DNA. Challenges and Advances In Computational Chemistry And Physics. Dordrecht, The Netherlands.: Springer; 2004.

[45] Laing C, Schlick T. Computational approaches to RNA structure prediction, analysis, and design. Curr Opin Struct Biol. 2011;21(3):306-318. doi:10.1016/j.sbi.2011.03.015.

[46] Russell R. Introduction and Overview. In: Russell R, editor. Biophysics of RNA Folding. New York, NY: Springer New York; 2013. p. 1-10.

[47] Flores SC, Jonikas M, Bruns C, Ku JP, Schmidt J, Altman RB. Methods for Building and Refining 3D Models of RNA. In: Leontis N, Westhof E, editors. RNA 3D Structure Analysis and Prediction. Nucleic Acids and Molecular Biology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 143-66.

[48] Leontis N, Westhof E. RNA 3D Structure Analysis and Prediction Nucleic Acids and Molecular Biology vol 27. Berlin Heidelberg: pringer-Verlag 2012.

[49] Rother K, Rother M, Boniecki M, Puton T, Tomala K, Łukasz P et al. Template-Based and Template-Free Modeling of RNA 3D Structure: Inspirations from Protein Structure Modeling. In: Leontis N, Westhof E, editors. RNA 3D Structure Analysis and Prediction. Nucleic Acids and Molecular Biology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 67-90.

[50] Rother M, Milanowska K, Puton T, Jeleniewicz J, Rother K, Bujnicki JM. ModeRNA server: an online tool for modeling RNA 3D structures. Bioinformatics. 2011;27(17):2441-2442. doi:10.1093/bioinformatics/btr400.

[51] Rother M, Rother K, Puton T, Bujnicki JM. RNA tertiary structure prediction with ModeRNA. Brief Bioinform. 2011;12(6):601-613. doi:10.1093/bib/bbr050.

[52] Boniecki MJ, Lach G, Dawson WK, Tomala K, Lukasz P, Soltysinski T et al. SimRNA: a coarse-grained method for RNA folding simulations and 3D structure prediction. Nucleic Acids Res. 2016;44(7):e63. doi:10.1093/nar/gkv1479.

[53] Magnus M, Boniecki MJ, Dawson W, Bujnicki JM. SimRNAweb: a web server for RNA 3D structure modeling with optional restraints. Nucleic Acids Res. 2016;44(W1):W315-W319. doi:10.1093/nar/gkw279.

[54] Piatkowski P, Kasprzak JM, Kumar D, Magnus M, Chojnowski G, Bujnicki JM. RNA 3D Structure Modeling by Combination of Template-Based Method ModeRNA, Template-Free Folding with SimRNA, and Refinement with QRNAS. RNA Structure Determination: Methods and Protocols. 2016. p. 217-35.

[55] Capriotti E, Norambuena T, Marti-Renom MA, Melo F. All-atom knowledge-based potential for RNA structure prediction and assessment. Bioinformatics. 2011;27(8):1086-1093. doi:10.1093/bioinformatics/btr093.

[56] Chen VB, Arendall WB, 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GJ et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr. 2010;66(Pt 1):12-21. doi:10.1107/S0907444909042073.

[57] Davis IW, Leaver-Fay A, Chen VB, Block JN, Kapral GJ, Wang X et al. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. 2007;35(Web Server issue):W375-83. doi:10.1093/nar/gkm216.

[58] Griffiths-Jones S. RALEE--RNA ALignment editor in Emacs. Bioinformatics. 2005;21(2):257-259. doi:10.1093/bioinformatics/bth489.

[59] Skinner J, Bond W. Sublime Text 3 3.1.1 ed. Darlinghurst NSW 2010, Australia: Sublime HQ Pty Ltd; 2018.

[60] Darty K, Denise A, Ponty Y. VARNA: Interactive drawing and editing of the RNA secondary structure. Bioinformatics. 2009;25(15):1974-1975. doi:10.1093/bioinformatics/btp250.

[61] Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF et al. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. J Struct Biol. 2012;179(3):269-278. doi:10.1016/j.jsb.2011.09.006.

[62] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-1612. doi:10.1002/jcc.20084.

[63] Schrödinger LLC. The PyMOL Molecular Graphics System. 2.1 ed. New York, NY: Schrödinger LLC; 2018.

[64] BIOVIA DS. Discovery Studio Modeling Environment, Release 2017, Dassault Systèmes, 2016. San Diego2016.

[65] Petrov AI, Zirbel CL, Leontis NB. WebFR3D--a server for finding, aligning and analyzing recurrent RNA 3D motifs. Nucleic Acids Res. 2011;39(Web Server issue):W50-5. doi:10.1093/nar/gkr249.

[66] Zhong C, Tang H, Zhang S. RNAMotifScan: automatic identification of RNA structural motifs using secondary structural alignment. Nucleic Acids Res. 2010;38(18):e176. doi:10.1093/nar/gkq672.

[67] Zhong C, Zhang S. RNAMotif ScanX: a graph alignment approach for RNA structural motif identification. RNA. 2015;21(3):333-346. doi:10.1261/rna.044891.114.

[68] Musyoka TM, Kanzi AM, Lobb KA, Tastan Bishop O. Structure Based Docking and Molecular Dynamic Studies of Plasmodial Cysteine Proteases against a South African Natural Compound and its Analogs. Sci Rep. 2016;6:23690. doi:10.1038/srep23690.

[69] Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res. 2005;33(Web Server issue):W363-7. doi:10.1093/nar/gki481.

[70] Noller HF, Green R, Heilek G, Hoffarth V, Hiittenhofer A, Joseph S et al. Structure and function of ribosomal RNA. Biochem Cell Biol. 1995;73:997-1009

[71] Eisen J, Coil D. Fact Sheet: Ribosomal RNA (rRNA), the details In: Lester E, editor. microBEnet: the microbiology of the Built Environment network: microBEnet; 2018.

[72] Mwangi, Harrison N.; Muge, Edward K.; Wagacha, Peter W.; Ndakala, Albert; Mulaa, Francis J. 2021. "Methods for Identifying Microbial Natural Product Compounds that Target Kinetoplastid RNA Structural Motifs by Homology and De Novo Modeled 18S rRNA" *Int.* J. Mol. Sci. 22, no. 9: 4493. https://doi.org/10.3390/ijms22094493